ASSESSMENT OF THE CONTEMPORARY POPULATION STRUCTURE AND ADMIXTURE OF ATLANTIC SWORDFISH (*XIPHIAS GLADIUS* L.) VIA MIXED STOCK ANALYSIS AND BAYESIAN CLUSTERING OF MULTIPLE NUCLEAR SNPS GENOTYPED THROUGH HIGH RESOLUTION MELTING

A Dissertation

by

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ABSTRACT

North Atlantic and South Atlantic swordfish (*Xiphias gladius* L.) are currently managed as two stocks separated at 5°N. While previous studies of genetic population structure using both mitochondrial and nuclear DNA confirm two genetically distinct stocks, sampling coverage has not been uniform or representative of all areas and estimates of admixture in areas of contact have not been provided. In this study, we examined: 1) the applicability of high-resolution melting analysis (HRMA) in population genetic studies of non-model organisms, 2) the use of nuclear markers in Atlantic swordfish and the methodology whereby nuclear gene variation can be quickly screened, identified, and genotyped using short-amplicon (SA) HRMA and unlabeled probe (UP) HRMA, and 3) the use of HRMA to characterize nuclear markers to study the genetic population structure of Atlantic swordfish using representative samples of the entire basin to provide an estimation of population admixture by means of Bayesian individual assignment.

High resolution melting analysis (HRMA) is shown to be a highly sensitive, rapid, closed-tube genotyping method amenable to high throughput and, though until recently primarily confined to clinical studies, suitable for population studies in non-model species. Ten nuclear markers were genotyped primarily by SA- and UP-HRMA in North Atlantic (n=419), South Atlantic (n=296), and Mediterranean (n=59) swordfish. Comparisons of pairwise F_{ST}, AMOVA, PCoA, and Bayesian individual assignments were congruent with previous finding of three discrete populations with comparatively

low levels of estimated gene flow for a marine organism (F_{ST} = 0.039-0.126). Population admixture was identified and estimated in the Northeast Atlantic and appeared to be asymmetrical, with swordfish from the South Atlantic found among North Atlantic localities but no North Atlantic migrants identified in the South Atlantic. The Mediterranean boundary currently at the Strait of Gibraltar is found to extend west into Atlantic waters to approximately 8°W. Similarly, the boundary between North and South Atlantic swordfish should be revised to a line that extends north from 0°N 45°W to 25°N 45°W and from that position, as a nearly horizontal line, eastwards to the African coast. Finally, I show that Bayesian individual assignment using the developed marker set can be used for mixed stock allocation in the Northeast Atlantic.

DEDICATION

This dissertation is dedicated to my wife, Karrie, who has supported me emotionally and financially throughout my doctoral quest. This dissertation is the direct byproduct of your support, patience, love, and unequivocal belief in my abilities. We started this odyssey as newly expectant parents and now find ourselves outnumbered by three boys and yet I know that no matter the course of our future, as partners, we can summit the peaks and traverse the valleys. To my children, your unfathomable thirst for knowledge, though at times exhausting, has fueled my own pursuit of further light and knowledge. May you always question your assumptions and learn to take pleasure in the mysteries of our universe.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Biological background for Atlantic swordfish

The swordfish (*Xiphias gladius* Linnaeus 1758) is the sole member of the family Xiphiidae. While fossils of extant swordfish from Italy date to the middle Pliocene (~3 Ma) (Sorbini 1987), divergence of Xiphiidae from Istiophoridae is believed to have occurred pre-Eocene (>55 Ma) (Fierstine & Applegate 1974). Swordfish are morphologically distinguished from istiophorid billfishes (spearfish, sailfish, and marlin) by the absence of pelvic fins, a long and broadly flattened bill, large keels on each side of the caudal peduncle, and in adults; scales deeply embedded within the dermis; and absence of teeth (Govoni *et al.* 2004; Nakamura 1985; Palko *et al.* 1981). Swordfish possess numerous morphological and physiological adaptations to maintain brain, eye, and cardiac function in cold waters (Block *et al.* 1993; Carey 1982; Dickson & Graham 2004; Galli *et al.* 2009), which enable longer deeper daily vertical migrations compared to istiophorids and scombrids (Brill *et al.* 1993; Dewar *et al.* 2011; Horodysky *et al.* 2007; Musyl *et al.* 2003; Prince *et al.* 2010). Consequently, the distribution of swordfish in the world oceans is broader than istiophorids extending to 50°N to 50°S (Nakamura 1985).

Atlantic swordfish segregate by size and gender with larger individuals, usually females, able to inhabit colder waters at higher latitudes which are utilized as feeding

grounds (Palko *et al.* 1981). Mature females return to tropical waters to spawn where sea surface temperatures (SST) are 24°C or greater (Govoni *et al.* 2000). In the northwestern Atlantic spawning occurs year round in the Gulf of Mexico, Straits of Florida, Caribbean Sea, southern edge of the Sargasso Sea, and in the waters of the Gulf Stream as far north as Cape Hatteras (Govoni *et al.* 2000; Neilson *et al.* 2007; Palko *et al.* 1981). In the South Atlantic spawning occurs year round in an equatorial band west of 10°W between 5°N and 5°S, as well as between 15-35°S and 20-40°W (Alvarado Bremer *et al.* 2005a; Mejuto & García-Cortés 1997). By contrast, the reproductive season and distribution in the Mediterranean is more protracted, occurring only from June to August, when water temperatures reach 24°C, in geographically discrete spawning grounds (Palko *et al.* 1981; Tserpes *et al.* 2008).

Conventional tag-recapture experiments on Atlantic swordfish have been conducted primarily in the fishing grounds of the Northwest Atlantic and thus may not be representative of the movements throughout the entire basin. These data, however, suggest that both trans-equatorial and trans-Atlantic movements are rare (summary in Neilson *et al.* 2007), with most of the swordfish tagged in the Northwest Atlantic feeding grounds recaptured in putative spawning grounds. Although the number of pop-up satellite archival tags (PSATs) is limited, it provides additional evidence of seasonal spawning-to-feeding ground migrations in the Northwest Atlantic (Neilson *et al.* 2009; Sedberry & Loefer 2001). PSATs also indicate that swordfish spend the night near-surface and descend slowly prior to sunrise to depths >500 m where they spend the

majority of the day foraging in the deep sound scattering layer before returning to the surface mixed layer after sunset (Dewar *et al.* 2011).

It is during the night, when they are close to the surface, that commercial longline fisheries target swordfish. Atlantic swordfish is managed by the International Commission for the Conservation of Atlantic Tunas (ICCAT) as three separate stocks; the North Atlantic and South Atlantic separated at 5°N, and the Mediterranean separated at the Strait of Gibraltar. In 2010 the total allowable catches for the North Atlantic and South Atlantic were 13,700 tons and 15,000 tons, yet only 12,154 tons and 12,566 tons were actually captured, respectively (ICCAT 2010). Currently the Atlantic swordfish stocks are neither considered overfished or experiencing overfishing as fishing pressure on Atlantic swordfish has decreased over the last decade. However, a better understanding of the population structure of the North and South Atlantic stocks is still needed by fisheries managers for management decisions and may prove applicable in other large pelagic fishes.

Current status of swordfish genetic population structure in Atlantic

Atlantic swordfish genetic population structure has been investigated using allozymes (Grijalva-Chon *et al.* 1996; Pujolar *et al.* 2002), mitochondrial DNA (mtDNA)(Alvarado Bremer *et al.* 1995; Alvarado Bremer *et al.* 2005a; Alvarado Bremer *et al.* 1999; Alvarado Bremer *et al.* 1996; Chow *et al.* 1997; Chow & Takeyama 2000; Rosel & Block 1996), and nuclear DNA (nDNA) both targeting gene loci (Alvarado Bremer *et al.* 2007; Chow *et al.* 2007; Chow & Takeyama 2000; Grieg *et al.* 2000) and

microsatellites (Kasapidis *et al.* 2007; Kasapidis *et al.* 2009; Kasapidis *et al.* 2008; Kotoulas *et al.* 2007; Reeb *et al.* 2003). All these studies are concordant regarding the pronounced genetic differentiation of Mediterranean and Atlantic swordfish populations. Further, analyses of the mtDNA D-loop (CR-I) identified the presence of two highly divergent clades that have asymmetrical distribution worldwide and reveal substantially lower levels of haplotypic diversity in the Mediterranean Sea compared to any other basin (Alvarado Bremer *et al.* 1995; Alvarado Bremer *et al.* 1996; Pujolar *et al.* 2002; Rosel & Block 1996). The lower haplotypic diversity in the Mediterranean Sea and the reciprocal monophyly of mtDNA lineages with respect to North Atlantic swordfish, has been interpreted as the consequence of dramatic reductions in population size and vicariance, followed by population expansion and secondary contact associated with Pleistocene glacial and inter-glacial eustatic events (Alvarado Bremer *et al.* 2005b). The heterogeneous distribution of Clade I and Clade II mtDNA lineages, and of alpha Clade I lineages that are more common in the North Atlantic, significantly differentiates the North Atlantic and the South Atlantic populations (Alvarado Bremer *et al.* 2005a; Alvarado Bremer *et al.* 1996; Alvarado Bremer *et al.* 2005b).

While mtDNA has been useful to demonstrate the division of Atlantic swordfish into at least two populations, because of its uniparental mode of inheritance it may reflect the behavior of females and not of the entire population thereby limiting its use to determine the levels of population admixture. Studies using nDNA genetic markers have also found significant differentiation among North Atlantic, South Atlantic, and Mediterranean populations (Alvarado Bremer *et al.* 2007; Chow *et al.* 2007; Chow &

Takeyama 2000; Grieg *et al.* 2000) that are for the most part concordant with mtDNA patterns of differentiation. Furthermore, the PCR-RFLP analysis of a single nucleotide polymorphism in intron-4 of the calmodulin gene (*CaM*) identifies a significant break in allele frequencies between the North and the South Atlantic, leading to Chow *et al.* (2007) to propose a change in the position of the management boundary from 5°N to 15°N. This latter study has two large limitations. First, while admixture zones were identified by Chow *et al.* (2007) the use of a single marker lacks the power for individual assignment in mixed stock analysis (Morin *et al.* 2009). Second, the sampling coverage was not sufficient to properly determine the correct location of the boundary between North and South Atlantic populations.

To address these deficiencies, Kasapidis *et al.* (2008) assessed the utility of multiple microsatellite loci for Bayesian individual assignment of swordfish to their corresponding stocks. However, the estimates of genetic population differentiation obtained in that study were an order of magnitude lower than results obtained with mtDNA and nDNA, and thus not concordant with the levels of genetic heterogeneity of North and South Atlantic swordfish. The absence of a signal of differentiation microsatellites data could be explained by several reasons. For instance, if microsatellite mutation rate is high with strong allele constraints in a population with a large effective population size, size homoplasy can bias estimates of differentiation (Estoup *et al.* 2002). In addition, microsatellite heterozygosities are reportedly higher in marine fishes than freshwater or anadromous fishes (DeWoody & Avise 2000) and an inverse relationship between F_{ST} and microsatellite polymorphism is reported in pollock

(O'Reilly *et al.* 2004). A multilocus analysis of nDNA markers may be better suited for Bayesian individual assignment in swordfish. However, in order to be able to assess levels of variation contained in nDNA sequences, a methodology for rapid nuclear variation screening and genotyping is needed as well as the development of an approach to efficiently identify additional nuclear markers to be screened with fast genotyping approaches.

Organization of chapters

This dissertation is organized into four additional chapters that follow. Chapter II describes the applicability of HRMA for use in population genetic studies of non-model species. As HRMA was previously confined to use in clinical and diagnostic laboratories, and we were uncertain whether the type of tissue, isolation methodology, and/or DNA template quality would preclude the use of the highly sensitive technology as there were no reports of HRMA usage in population genetic studies. Chapter III outlines the methodology used to screen nuclear variation with HRMA, short-amplicon (SA) and unlabeled probe (UP) HRMA genotyping assay development and subsequent high-throughput genotyping, and the characterization of the nuclear markers developed for Atlantic swordfish genetic stock structure analysis. The applicability of the methodology is also outlined for use in other non-model nuclear variation studies. Chapter IV assesses the genetic population structure Atlantic swordfish based on 774 swordfish from 18 localities representative of North Atlantic, South Atlantic, and Mediterranean populations characterized for 10 nuclear loci. Bayesian clustering

analysis and individual assignment is evaluated for potential mixed stock analysis and population admixture estimation. Finally, the general conclusions of this dissertation are given in Chapter V including overall results, findings, implications, and recommendations.

CHAPTER II

HIGH-RESOLUTION MELTING ANALYSIS (HRMA): A HIGHLY SENSITIVE INEXPENSIVE GENOTYPING ALTERNATIVE FOR POPULATION STUDIES*

Introduction

High-resolution melting analysis (HRMA) is a highly sensitive molecular method for mutation scanning and genotyping confined to date to clinical and diagnostic studies (Lee *et al.* 2008; Reed *et al.* 2007; Vandersteen *et al.* 2007). HRMA detects single nucleotide polymorphisms (SNP's) and small deletions in a fragment of amplified DNA by comparing fluorescence as a function of temperature. Alleles produce distinct melting curves that can be compared to reference samples. The use of a saturating DNA dye enhances the detection of heteroduplexes (Graham *et al.* 2005) that can be identified through changes in melting curve shape (Palais et al., 2005). Variations of HRMA have been developed to enhance resolution. In small amplicon (SA) HRMA the 3'ends of a primer set are placed at a very short distance from the informative SNP (Gundry *et al.* 2008). The small size (40-60 bp) of the resulting amplicon enhances the melting temperature (T_m) differences among homozyogous genotypes. Alternatively, unlabeled probe (UP) HRMA can be used when multiple informative SNPs are present within a longer stretch of sequence, but also when low GC content or the presence of polymorphisms prevents the placement of SA-HRMA primers. In UP-HRMA the T_m of the unlabeled probe, and not of the entire amplicon, is used for genotyping (Gundry *et al.* 2008; Liew *et al.* 2007; Poulson & Wittwer 2007). The entire procedure from PCR to

*Reprinted with permission from "High-resolution melting analysis (HRMA): a highly sensitive inexpensive genotyping alternative for population studies" by Smith, B.L, Lu, C.P., Alvarado Bremer, J.R., 2010, *Molecular Ecology Resources*, 10, 193-196, Copyright 2009 by Copyright Clearance Center.

scoring is completed within 15-20 minutes as a single closed-tube assay. Based on all these attributes a wide use of this technique would be expected, however a literature review as of April 2009 revealed no hits of HRMA in wild populations despite the increasing importance of SNPs in this field. Here we use HRMA to genotype alleles in swordfish populations.

Methods

Swordfish (*Xiphias gladius* L.)(n=121) previously sampled from the North Atlantic, South Atlantic, Mediterranean, Hawaii, and Chile (Alvarado Bremer *et al.* 2006; Alvarado Bremer *et al.* 2005a) were used for HRMA. DNA was isolated from a small piece (≈4µg) of tissue (liver, heart, muscle, or fin clips) using a Proteinase K digestion followed by ETOH precipitation without organic extractions (Grieg 2000). Polymerase chain reactions (PCR) for SA-HRMA were performed in 10µL volumes in LightCycler capillaries (Roche Diagnostics) containing: 0.2M Trehalose, 250µg/mL BSA, 1X LCGreen Plus (Idaho Technology), 1X PCR Buffer with 3.0mM $MgCl_2$ (Idaho Technology), 200µM each dNTP, 0.5U of Taq polymerase (Lucigen), 10ng DNA template, and 0.25µM of each primer. UP-HRMA required an asymmetric PCR with a reverse primer and an unlabeled 3'-phosphorylated probe at 0.5 µM each, and a forward primer at 0.075 µM, with all other reagents as above. Reactions were overlaid with mineral oil to prevent evaporative losses ensuring melting profile uniformity. Thermocycling was carried on a RapidCycler II (Idaho Technology) with HRM primers as outlined in **Table 1**. Prior to melting analysis in a HR-1 instrument (Idaho

Technology), samples were heated to 94ºC and cooled rapidly to 40ºC to maximize heteroduplex formation.

Results and discussion

Loci CaM, ARP and Mlc2 are bi-allelic. CaM and ARP were genotyped using SA-HRMA, revealing all three possible genotypes, respectively (**Figure 1A-B**). Because the regions flanking the informative SNP in Mlc2 were not optimal for SA-HRMA primer design, an unlabeled probe producing three distinct melting profiles was designed (**Figure 1C**). Locus ldh-A also required an unlabeled probe that contained two polymorphic loci which define three-alleles and six genotypes (**Figure 1D**). In all instances, the alleles scored with HRMA matched the genotypes from DNA sequences (Genbank AF069912-AF069913, FJ890938-FJ890941, FJ911901-FJ911904).

Population genetic studies targeting SNP's have employed a variety of genotyping methods (reviewed in Kwok 2003), affected by one or more of the following shortcomings associated primarily with post-PCR manipulation: false negatives (e.g., endonuclease inactivity), time consuming with numerous steps and platforms (e.g., gels, enzymatic reactions and cleaning procedures), and or costly (e.g., fluorescently labeled probes). In addition, informative SNP's may not coincide with an endonuclease recognition site, or an allele size polymorphism may not exist within an amplicon. HRMA circumvents most of these limitations. One concern with HRMA is that ionic

Table 1. New HRMA primers and PCR profiles used in this study. Amplicon length was minimized to maximize melting temperature differences for genotyping. Polymorphic loci located in the unlabeled probes are underlined and in bold.

Locus	HRMA Primer Name	HRMA Primer Sequence	Thermocycling Profile
CaM (Chow & Takeyama 2000)*	CALMex4-INT-F	5'-TGCACACATTTGATCCTGTGAC-3	94°C 1 min, followed by 55 cycles at 94°C 0 s, 60°C 0 s, 66°C 10 s
	CALMex5-INT-R	5'-GTAGCCATTTCCGTCCTGGA-3'	
ARP (Naruse *et al.* 2004)*†	Olb03.10-HRM-F	5'-GCTACTCCTGTCTGTCTAAATC-3'	94°C 1 min, followed by 55 cycles at 94°C 0 s, 50°C 0 s, 72°C 10 s
	Olb03.10-HRM-R	5'-GTCCCTAGCTGCCGGAA-3'	
ldh-A (Grieg 2000)*	ldhA-HRM-SwoF	5'-AGCAAGCCCTGAACTTC-3'	94°C 1 min, followed by 55 cycles at 94°C 0 s, 66°C 10 s
	ldhA-HRM-SwoR2	5'-GCCGAAAGGACAGGGTGAGC-3'	
	ldhA-HRM-Probe	5'-ATTCATC**C**TGTTGATTAGTTTACAAAACATA**A**TGTACAT/3Phos/-3'	
Mlc2 (Atarhouch *et al.* 2003)*	Mlc2c-F	5'-CTGTCGCACTGGGTGGTCA-3'	94°C 1min, followed by 55 cycles at 94°C 0 s, 55°C 0 s, 72°C 10 s
	Mlc2b-R	5'-CCAACACTCACTCCTGAATTGG-3'	
	Mlc2-HRM-Probe	5'-GTAGCGGGAACTT**G**TGCTCAAACACCT/3Phos/-3'	

*Citations provided for the primers used to obtain DNA sequences and identify SNPs targeted with HRMA primers. †Locus Olb03.10 is orthologous to locus ARP.

strength and template concentration could alter the amplicon T_m. This may explain why HRMA has been confined to clinical or diagnostic studies, where uniformity in DNA template quality and quantity is generally higher than samples collected in the field. In here, however, DNA was isolated from several tissues sources without phenol-chloroform extractions, and regardless of the source, the melting profile for each allele was unambiguous and repeatable (**Figure 1**). While we relied on the HR-1 instrument for melting analyses, the sensitivity and effectiveness of other platforms have been evaluated (Herrmann *et al.* 2006; Herrmann *et al.* 2007). The high throughput, low cost, and high resolution to discriminate alleles makes HRMA a desirable genotyping tool for studying wild populations.

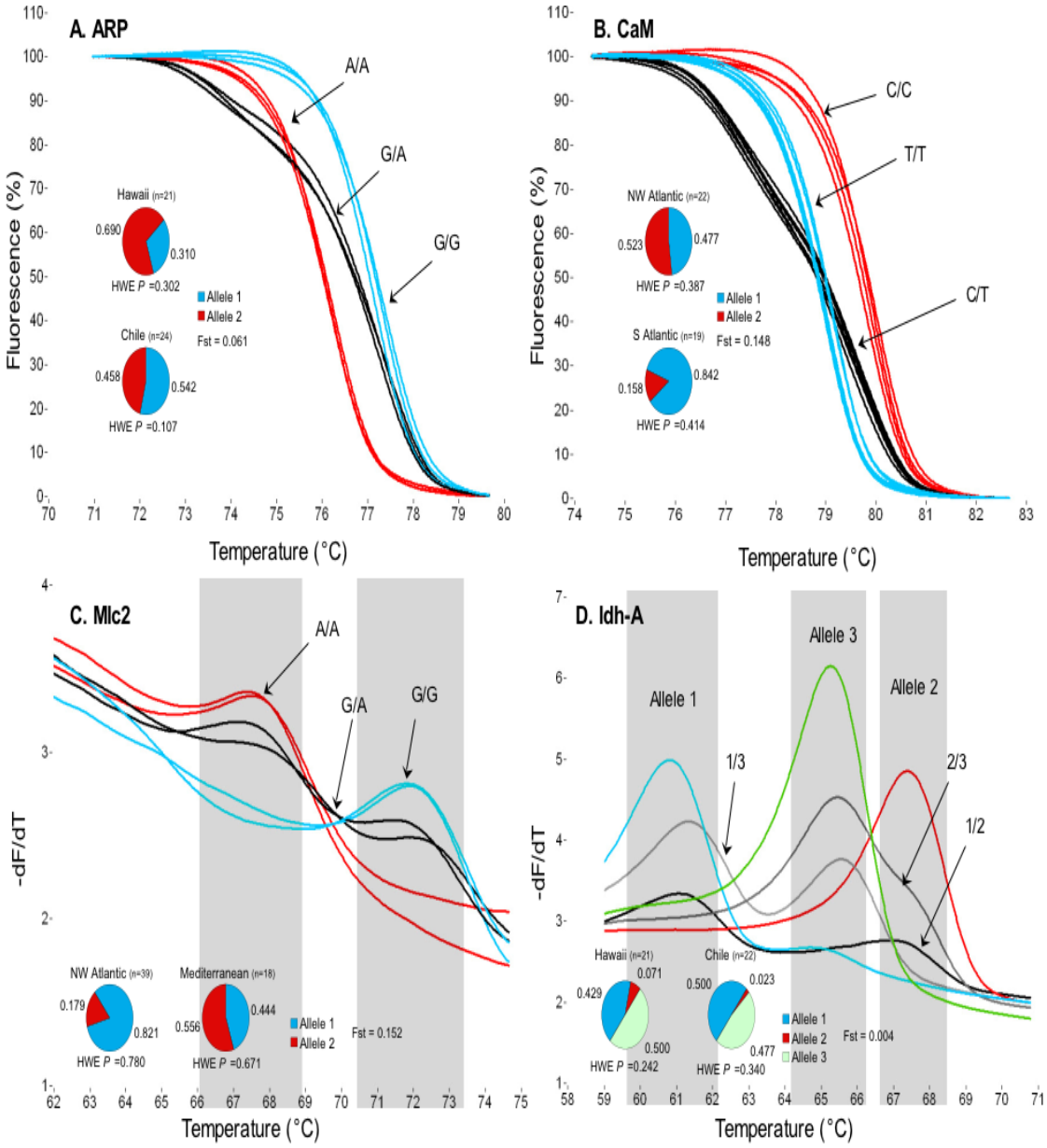

Figure 1. Genotyping swordfish populations using HRMA. Each graph (A-D) depicts melting curves from multiple individuals for the corresponding locus. Pie charts (insets) summarize the allele frequencies for different samples and the respective pairwise F_{st} value. All samples are in HWE (P>0.05). In the normalized curves of ARP (A) and CaM (B) using SA-HRMA, homozygous genotypes are distinguished by T_m, whereas heterozygous individuals by melting curve shape. In UP-HRMA (C-D), negative derivative plots of fluorescence with respect to temperature (-dF/dT) were obtained from the melting curves of the probes for Mlc2 and ldh-A (Table 1). The Mlc2 unlabeled probe targets an A/G SNP. The ldh-A unlabeled probe targets two polymorphic loci (T/C/A and A/C) that define the three most common alleles described in Alvarado Bremer et al. (2006) for Pacific swordfish. Homozygous individuals have one peak and heterozygous individuals two peaks (e.g., genotype 1/3) within the corresponding melting points.

CHAPTER III

METHODOLOGICAL STREAMLINING OF SNP DISCOVERY AND GENOTYPING VIA HIGH-RESOLUTION MELTING ANALYSIS (HRMA) IN NON-MODEL SPECIES

Introduction

Advances in genomic technologies provide additional resources for genetic marker development in non-model marine species for ecological and evolutionary studies (Allendorf *et al.* 2010). Such progress coincides with the increasing adoption of single nucleotide polymorphisms (SNPs) in multi-locus studies. While during the last two decades most population studies have utilized microsatellite loci, application of SNPs have revealed lower associated genotyping errors, simpler mutation models, comparable differentiation power, higher amenability to automation and high through-put technologies, and increased amplification success with low-quality and ancient DNA (Morin *et al.* 2004; Morin *et al.* 2009; Ryman *et al.* 2006).

Studies of intra-specific variation contained in coding and non-coding regions benefit from developments that allow a rapid screening of variation and subsequent genotyping. New technologies in genotyping and next generation sequencing (NGS) for SNP screening are increasing both speed and throughput while reducing costs (Seeb *et al.* 2011a). While NGS technologies have wide implications for rapid and abundant SNP discovery and the development of SNPs in non-model species continues to progress

(e.g. Helyar *et al.* 2012; Nielsen *et al.* 2012; Nielsen *et al.* 2011; Ogden *et al.* 2012), without genomic references *de novo* assembly for targeted or closely related species is still difficult and expensive (Everett *et al.* 2011).

High resolution melting analysis (HRMA) is a highly sensitive, closed-tube molecular mutation scanning and genotyping method routinely applied in clinical and diagnostic studies and is easily amenable for high throughput (Reed *et al.* 2007; Vandersteen *et al.* 2007; Zhou *et al.* 2005). In HRMA the fluorescence of a saturating DNA dye (e.g. LCGreen+, EvaGreen, etc.) is measured as a function of melting temperature of amplified DNA. Melting curve profiles and temperatures (T_m) are diagnostic of SNPs and small deletions (Graham *et al.* 2005). Small amplicon (SA) and unlabeled probe (UP)-HRMA help alleviate genotyping error (here termed 'genotype masking') caused by multiple or base-pair neutral variants (Gundry *et al.* 2008). In SA-HRMA the 3' ends of primers flank the targeted SNP(s) to minimize genotype masking and enhance the melting temperature differences (ΔT_m) between alleles (Gundry *et al.* 2008; Smith *et al.* 2010). In UP-HRMA the T_m of the unlabeled probe, and not of the entire amplicon, is scored to eliminate genotype masking and provides the ability to genotype multiple SNP's contained in the probed segment (Liew *et al.* 2007; Smith *et al.* 2010). Due to the high sensitivity of HRMA the effects of ionic strength, template quality, and other template chemistry parameters have been investigated (reviewed in (Wittwer 2009)). Concerns of HRMA suitability in wild population studies, as opposed to clinical studies where template quality and chemistry are easily controlled, were addressed by Smith et al. (Smith *et al.* 2010) who demonstrated that genotyping results

using DNA template isolated from different tissues without organic extractions were unambiguous and repeatable. The use of HRMA to study wild fish populations has been published recently, although primarily in SNP screening (McGlauflin *et al.* 2010; Seeb *et al.* 2011b; Smith *et al.* 2010; Wetten *et al.* 2012).

In marine species there is need for better integration of population genetic data with resource management practices (Waples *et al.* 2008). This is particularly true in Atlantic and Mediterranean swordfish (*Xiphias gladius*) populations where genetic stock structure studies are beginning to investigate potential regions of admixture (Chow *et al.* 2007; Smith & Alvarado Bremer 2010). Using swordfish as an example, we provide a rapid and low-cost workflow, amendable to other species, aimed to facilitate the development HRMA assays for SNP screening and genotyping.

Materials and methods

Nuclear gene primers design and preliminary SNP screening

Initial nuclear gene primers were either 1) obtained from published population studies of teleost species targeting polymorphic nuclear genes, or 2) designed from consensus alignments of teleost DNA sequences from GenBank using Primer3 (Rozen & Skaletsky 2000) (**Table 2**). DNA of 774 adult swordfish from the North Atlantic (NA) (n=419), South Atlantic (SA) (n=296), and Mediterranean (MED) (n=59) were isolated from tissue using a Proteinase K digestion followed by ethanol precipitation without organic extractions (Grieg 2000). Preliminary PCRs of 30 swordfish, ten from each

Table 2. Details for HRMA primer development of ten nuclear genes and the results of SNP screening Atlantic and Mediterranean swordfish populations.

Locus name	Gene	Genbank accession #	Source	Primer Name	Primer sequence (5'->3')	e^{-value}	T_A (°C)	Exons	Introns	FL (bp)	SNPs	Indels
SRP54	Signal recognition particle 54	JX042451	NM200988, BT044934, BT027457, CR681634	SRP54-F1	GAA CAC ATT GAT GAC TTT GAG CC	$1.0E^{-18}$	63	3	2	878	6	-
				SRP54-R1	TGA TGT TCT GAA ACT GCT CGT ACA T							
ATPsβ	ATP synthase beta-subunit	JX042449	BC095620, AB208024, AB203582, FJ208949, BT027345	ATPsBeta-F1	GGG AAA TGA CTT GTA CCA TGA GAT GAT	$3.0E^{-66}$	60	4	3	~950	20	2
				ATPsBeta-R3	GTC ATC AGA TAC TTC CCC ACC G							
CaM	Calmodulin	AF069912-AF069913	Chow 1998	CALMex4-F	CTG ACC ATG ATG GCC AGA AA	$9.0E^{-33}$	54	2	1	504	1	-
				CALMex5-R	GTT AGC TTC TCC CCC AGG TT							
ARP	Acidic ribosomal phosphoprotein P0	FJ890940-FJ89890941	Naruse et al 2004	OLb03.10-F	TAT CCA AAA TGC TTC ATC GTG GGA G	$3.0E^{-69}$	50	2	1	496	3	2
				OLb03.10-R	AGC AGC AGA TCC CTG ACT TCA GTC A							
GpHr	Golgi pH regulator	JX042450	Devlin et al 1994	OTY1-Y1	GAT CTG CTG GCT GGA TTT GG	$1.0E^{-27}$	50	2	2	372	6	-
				OTY1-Y2	CCA GCG ATG GTT TGT TTG AG							
ANT	Adenine nucleotide translocator	JX042446	BT026849, BT047290, AB240542, BC085562, NM001160491, BT079926	ANT-F2	TAC TTC GCT GGT AAC CTG G	$3.0E^{-95}$	53	2	1	487	10	2
				ANT-R1	GAC TGC ATC CAT CAT ACG ACG ACG							
Mlc2	Myosin light chain	FJ890938-FJ890939	Atarhouch et al 2003	Mlc2B-F	CTG TCG CAC TGG GTG GTC A	$1.0E^{-30}$	52	3	2	465	10	1
				Mlc2-R	CTT CAC CGT CGA CCTCAC CAT G							
ldhA	Lactose dehydrogenase A	FJ911901-FJ911904	Grieg 2000	LDHA6F1	TAC ACT TCC TGG GCG ATC GGG ATG	$2.9E^{-47}$	54	2	1	293	14	-
				LDHAR-SWO	GCT TGA GGA AGA CCT CGT CCT TCA C							
Act2a	Alpha skeletal actin 2	JX042448	Atarhouch et al 2003	ACT2-F	GCT ATA ACC CTC GTA GAT GGG CAC	$3.0E^{-69}$	54	2	1	341	8	-
				ACT2-R	ATC TGG CAC CAC ACC TTC TAC AA							
AldB	Aldolase B	JX042447	Grieg 2000	Ald1-5'	TGT GCC CAG TAT AAG AAG GAT GG	$2.0E^{-74}$	54	2	1	321	-	1 SSR
				Ald2-3'	CCC ATC AGG GAG AAT TTC AGG CTC CAC AA							

Abbreviations: T_A: locus-specific annealing temperature, FL: fragment length, e^{-value}: expected value of BLAST hits in GenBank

population, were conducted in 10 μL reactions containing 10 ng of genomic DNA, 1X Econotaq Plus Master Mix (Lucigen), 1X LCGreen+ (Idaho Technology), and 0.20 μM of each primer. Reactions were overlaid with mineral oil to ensure uniform melting among specimens by preventing evaporative losses that may unevenly alter salt concentrations (Smith *et al.* 2010). Thermal cycling was performed on a Lightcycler 480 Real-Time PCR system (Roche Diagnostics) with an initial denaturation of 10 min at 95 °C followed by 35 cycles denaturing for 10 s at 95 °C, annealing for 30 s at primer specific annealing temperature (T_A) (**Table 2**), and extension for 10 s at 72 °C. Prior to HRMA data acquisition, PCR products were denatured at 95 °C for 1 min and then rapidly cooled and incubated at 40 °C for 1 min. Data acquisitions (17 per °C) were collected between 60 and 95 °C at a melting ramp rate of 0.02 °C per sec. Specimens containing multiple amplicon melting profiles, indicative of genomic co-amplification (e.g. multiple peaks), were visualized via electrophoresis in 2% agarose gels pre-stained with ethidium bromide for secondary verification. If co-amplification of multiple loci was confirmed, or if primer-dimer artifacts were generated, PCR was further optimized to yield a single amplification product. If optimization was unsuccessful new primers were designed, optimized, and evaluated until single amplicons were produced. Single amplicons were screened for variant melting profiles, indicative of SNPs, using the LightCycler 480 Gene Scanning Software v. 1.5.0 SP1 (Roche Diagnostic). While we utilized the LightCycler 480, alternative platforms are available (see review in Herman et al. (Herrmann *et al.* 2006)).

Sanger sequencing of HRMA identified SNPs

Of the initial 30 individuals (10 from each population), HRMA templates with different homozygous and heterozygous melting profiles were sequenced to validate and provide initial base calls for SNPs. HRMA-templates were diluted 1:10 for post-PCR cleanup and sequenced in both directions with ABI BigDye terminator version 3.1 (Applied Biosystems) utilizing a ST_eP fast cycling protocol (Platt *et al.* 2007). Sanger sequencing were performed on a 3130 DNA Analyzer (Applied Biosystems) and multiple sequence alignments were carried out in Geneious v.5.6 (Kearse *et al.* 2012). Sequence alignments and electropherogram inspections were used to validate SNPs and evaluate HRMA-genotyping primer and probe design (**Figure 2**).

SA-HRMA genotyping assay design

SNPs, identified from initial HRMA and sequencing alignments at frequencies >5% among swordfish populations, were targeted for further new HRMA primer design following the workflow outlined in **Figure 2**. SA-HRMA primers were developed in non-variable regions flanking the SNP(s). Since amplicon length is directly proportional to the ΔT_m among alleles (Liew *et al.* 2007), SA-HRMA amplicons were designed to be <100 bp. This ensured that homozygous genotypes of two or more alleles were easily distinguished thus minimizing potential genotyping errors. SA-HRMA PCR optimization was conducted in 10 μL volumes following the same chemistry and thermo-cycling parameters described in preliminary SNP screening with the primers and T_A's given in Table 2. After optimization, samples (n=40) from the NA, MED, and SA

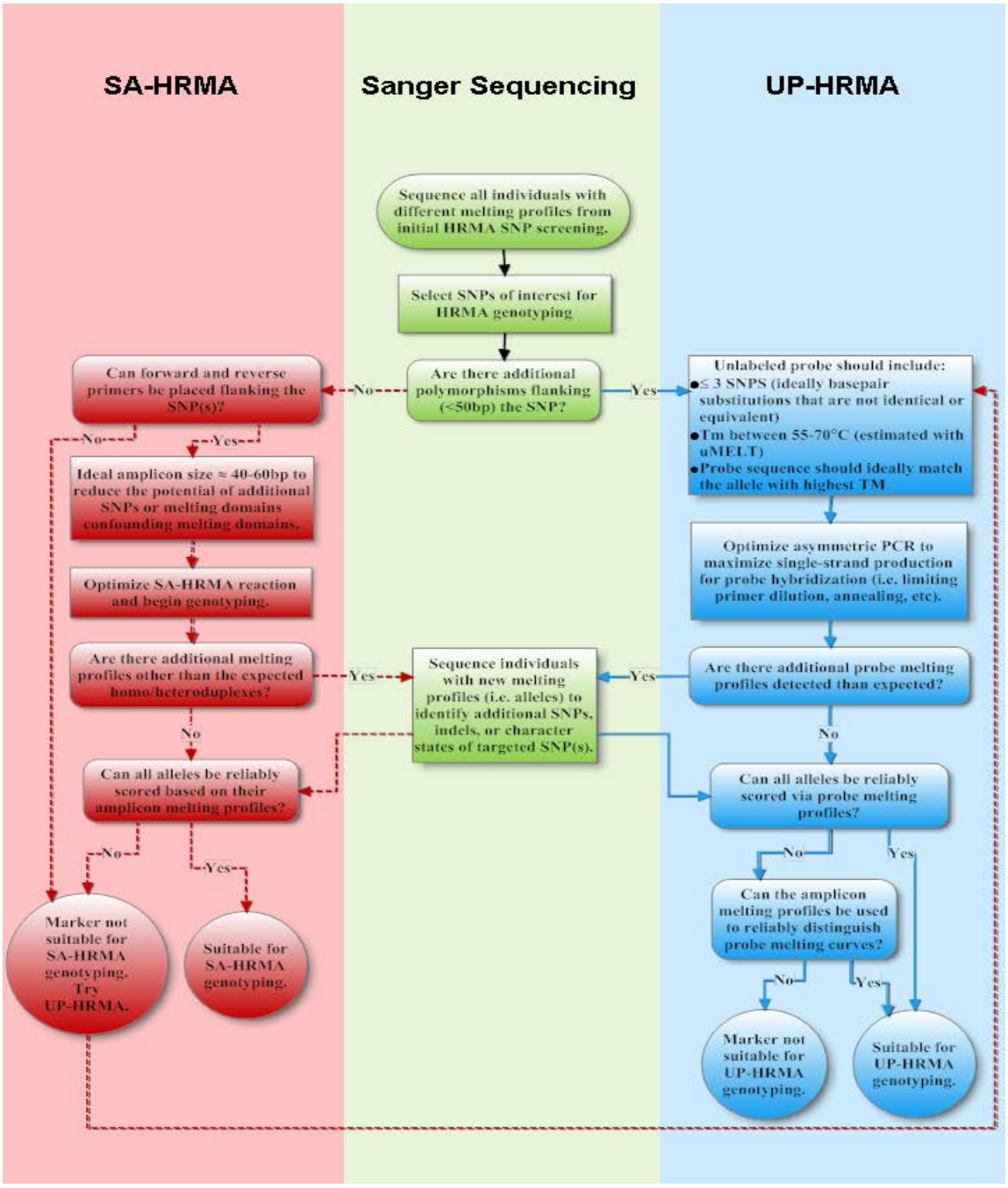

Figure 2. Workflow for HRMA assay design aimed to genotype wild populations. Workflow begins after initial HRMA SNP screening revealing polymorphisms. Individuals from representative samples with different melting profiles are sequenced via Sanger sequencing (green) to validate SNPs for subsequent SA-HRMA (red) primer design or UP-HRMA (blue) primer and probe design.

populations were then genotyped via SA-HRMA to validate the assay and identify additional melting curves (i.e. SNPs). Newly identified SNPs were validated via Sanger sequencing. SA-HRMA genotyping assays were retained when all alleles could be reliably scored. If SA-HRMA genotyping assays were not diagnostic, one of three courses of action was followed: 1) unlabeled probes were designed for UP-HRMA, 2) alternative non-HRMA genotyping methods were pursued, or 3) the SNP was abandoned (**Figure 2**). For the retained SA-HRMA assays, a total of 774 individuals were genotyped.

UP-HRMA genotyping assay design

UP-HRMA probes and primers (**Table 3**) were designed when 1) SNP flanking regions were not suitable for SA-HRMA primer placement; 2) if SA-HRMA genotyping was not diagnostic; or 3) when genotyping multiple SNPs (Figure 1). Unlabeled probes were designed to complement initial sequences containing only two to three SNPs. This constrained the potential for an unmanageable number of additional SNPs within the probe segment, not detected during initial screening, that may cause uncertainty when genotyping. Unlabeled probes were designed to match the haplotype with highest estimated T_m and with an overall probe T_m between 55-70 °C as estimated with uMELT^{SM} v2.0 (Dwight *et al.* 2011). Probe extension during PCR was prevented by 3' end phosphorylation. Asymmetric PCR, limiting primer dilution, and T_A were optimized to maximize single-strand production for probe melt genotyping. UP-HRMA 10 μL reactions followed the chemistry and thermocycling parameters given in **Table 3**

Table 3. SA-HRMA and UP-HRMA genotyping assay parameters and variability of swordfish samples collected in three geographic regions: North Atlantic (NA), Mediterranean (MED), and South Atlantic (SA).

										All 774 individuals						Adults from spawning areas					
										H_e			F_{IS} (W&C)			H_e			F_{IS} (W&C)		
Locus name	**Gene**	**HRMA**	**Primer**	**Primer/Probe Sequence (5'->3')**	**[Primer/ Probe] (µM)**	**T_A (°C)**	**Cycles**	**FL (bp)**	**A**	**NA (n=419)**	**MED (n=59)**	**SA (n=296)**	**NA (n=419)**	**MED (n=59)**	**SA (n=296)**	**NA (n=49)**	**MED (n=59)**	**SA (n=42)**	**NA (n=49)**	**MED (n=59)**	**SA (n=42)**
SRP54	Signal recognition particle 54	SA-HRMA	SRP54-saHRM-F1	CAA TCT ATC TCC GAA TGA CTG A	0.2	54	45	75	2	0.281	0.050	0.339	0.067	-0.018	-0.077	0.359	0.050	0.350	-0.005	-0.018	-0.281
			SRP54-saHRM-R1	CTG ATT TTT ATA CTT GTG TTA AGC CAA C	0.2																
ATPsBeta	ATP synthase beta-subunit	SA-HRMA	ATPsBeta-saHRM-F1	TTG ATA CTG ACT TCC TTC ATG T	0.2	54	45	60	2	0.490	0.493	0.466	0.061	0.046	0.007	0.493	0.493	0.459	0.057	0.046	0.079
			ATPsBeta-saHRM-R1	GAA TAC GAC CCA GCA GG	0.2																
CaM	Calmodulin	SA-HRMA	CALMex4-INT-F	TGC ACA CAT TTG ATC CTG TGA C	0.2	60	45	49	2	0.497	0.479	0.466	**0.116***	0.124	-0.068	0.497	0.479	0.172	-0.017	0.124	-0.093
			CALMex5-INT-R	GTA GCC ATT TCC GTC CTG GA	0.2																
ARP	Acidic ribosomal phosphoprotein P0	SA-HRMA	ARP-HRM-F	GCT ATC CCT GTC TGT CTA AAT C	0.2	50	45	41	2	0.447	0.324	0.484	**0.194***	-0.038	0.002	0.479	0.324	0.477	0.285	-0.038	0.163
			ARP-HRM-R	GTC CCT AGC TGC CGG AA	0.2																
GpHr	Golgi pH regulator	UP-HRMA	GpHr-HRM-F	GAT CTG CTG GCT GGA TTT GG	0.01	54	35	167	5	0.521	0.505	0.526	**-0.018***	-0.321	**0.063***	0.512	0.505	0.535	-0.105	-0.321	0.033
			GpHr-HRM-R	CCA GCG ATG GTT TGT TTG AG	0.2	44	25														
			GpHr-HRM-probe-allele2	GTT GAA TCT CTG TTT GCA TGG CAG TAC TCA ACA CAC AAG CTC AT /3Phos/	0.2																
ANT	Adenine nucleotide translocator	UP-HRMA	ANT-saHRM-4SNP-F	CTT ACT TTG GGG TCT ACG ACA C	0.2	58	35	252	7	0.532	0.539	0.497	0.035	-0.137	-0.019	0.522	0.539	0.458	0.154	-0.137	0.002
			ANT-R1	GAC TGC ATC ATC ATA CGA CGA CG	0.02	44	25														
			ANT-(UP)HRMA-Probe-R	AGG GTA CGT TTT CAC TAT CAA GCA TCA CAA AGC /3Phos/	0.2																
Mlc2	Myosin light chain	UP-HRMA	Mlc2E-F	TGC AGT GTA TTA AAA AGT ACA GTT CTT CC	0.02	54	35	111	3	0.398	0.500	0.365	**0.119***	-0.110	-0.017	0.320	0.500	0.338	0.180	-0.110	0.165
			Mlc2B-R	CCA ACACTC ACT CCT GAA TTG G	0.2	44	25														
			Mlc2-HRM-Probe	GTA GCG GGA ACT TGT GCT GCT CAA ACA CCT /3Phos/	0.2																
IdhA	Lactose dehydrogenase A	UP-HRMA	LDHSWOF-F	GTG GAA AGC CTC CTT AAG AAC CTG C	0.04	54	35	193	6	0.496	0.514	0.519	-0.088	-0.184	-0.029	0.468	0.514	0.489	-0.343	-0.184	0.135
			LDHSWO-HRM-R2	GCC GAA AGG ACA GGG TGA GC	0.2	44	25														
			LDH-HRM-allele2-unlabeledprobe	GAT ATT CAT CCT GTT GAT TAG TTT ACA AAA CAT AAT GTA CAT TCT AAA GA /3Phos/	0.2																

Abbreviations: T_A: locus-specific annealing temperature; Cycles: number of PCR cycles at the corressponding T_A (10 sec denaturation, 30 sec annealing, and 10 sec extension); FL: fragment length; A: number of alleles; H_e: expected heterozygosity; F_{IS}: fixation index from exact test for Hardy-Weinberg equilibrium, *P<0.05 and in bold.

and for HRMA screening (above). Locus-specific primer T_A's (**Table 3**) were utilized for the first 35 cycles of PCR, followed by an additional 25 cycles of PCR with a T_A of 44 °C to maximize single-strand product amplification (Alvarado Bremer et al. unpublished). Following UP-HRMA optimization additional samples (n=40) were then genotyped to validate the UP-HRMA assay and identify additional melting curves (i.e. SNPs). Products that generated additional probe melting curves were sequenced to verify base-calls for new SNPs within the probed segment. If the probe melting profiles of two or more alleles were indistinguishable, the corresponding melting profiles of the entire amplicons were inspected for characteristics that could be used to genotype the alleles. If both the probe and entire amplicon melting profiles were not diagnostic, either non-HRMA genotyping methods were pursued or the locus was abandoned. UP-HRMA genotyping assays were retained when all alleles could be reliably scored via the melting profiles (**see Figure 2**). For the retained UP-HRMA assays, a total of 774 swordfish were genotyped.

Results and discussion

HRMA SNP screening of 10 nuclear loci

HRMA screening and Sanger sequencing identified sequence polymorphisms in ten nuclear genes: acidic ribosomal phosphoprotein P0 (ARP), adenine nucleotide translocator (ANT), aldolase B (AldB), alpha skeletal actin 2 (Act2A), ATP synthase beta-subunit (ATPsβ), calmodulin (CaM), golgi pH regulator (GpHr), lactose dehydrogenase A (ldhA), myosin light chain (mlc2), and signal recognition particle 54

(SRP54). Amplicon lengths ranged between 300-950 bp and contained from one to three introns (**Table 2**). In total, 78 SNPs were identified during preliminary screening and later genotyping. Introns were nearly four times more variable than exons with 2.39 $\times 10^{-2}$ SNPs per intron base pair as compared to 6.38 $\times 10^{-3}$ SNPs per exon base pair. Transitions accounted for 54% of intron SNPs and 77% of exon SNPs. All exon SNPs were found to be synonymous after sequences were translated and aligned using BLASTX. SNPs differing at >5% among initial samples were selected as candidates for HRMA genotyping assay design. In most instances the entire amplicon contained more SNPs than was possible to genotype via initial HRMA. The CaM locus was the exception with the amplified fragment (504 bp) containing a single SNP. However, alternative CaM genotypes could not be reliably scored, in particular homozygous genotypes, due to the large size of the amplicon and corresponding small ΔT_m between alternative homozygote genotypes (**Figure 3A**). A SA-HRMA, targeting 49 bp, solved this genotyping problem (**Figure 3B**). While a number of factors influenced the success of initial HRMA screening (i.e. amplicon length, number of SNPs, intron/exon base-pair percentage, number of base-pair neutral SNPs, etc), a substantial reduction in sequencing effort for marker development was attained. Only those individuals identified by their distinct melting curves as new variants were sequenced, saving time, materials, and effort.

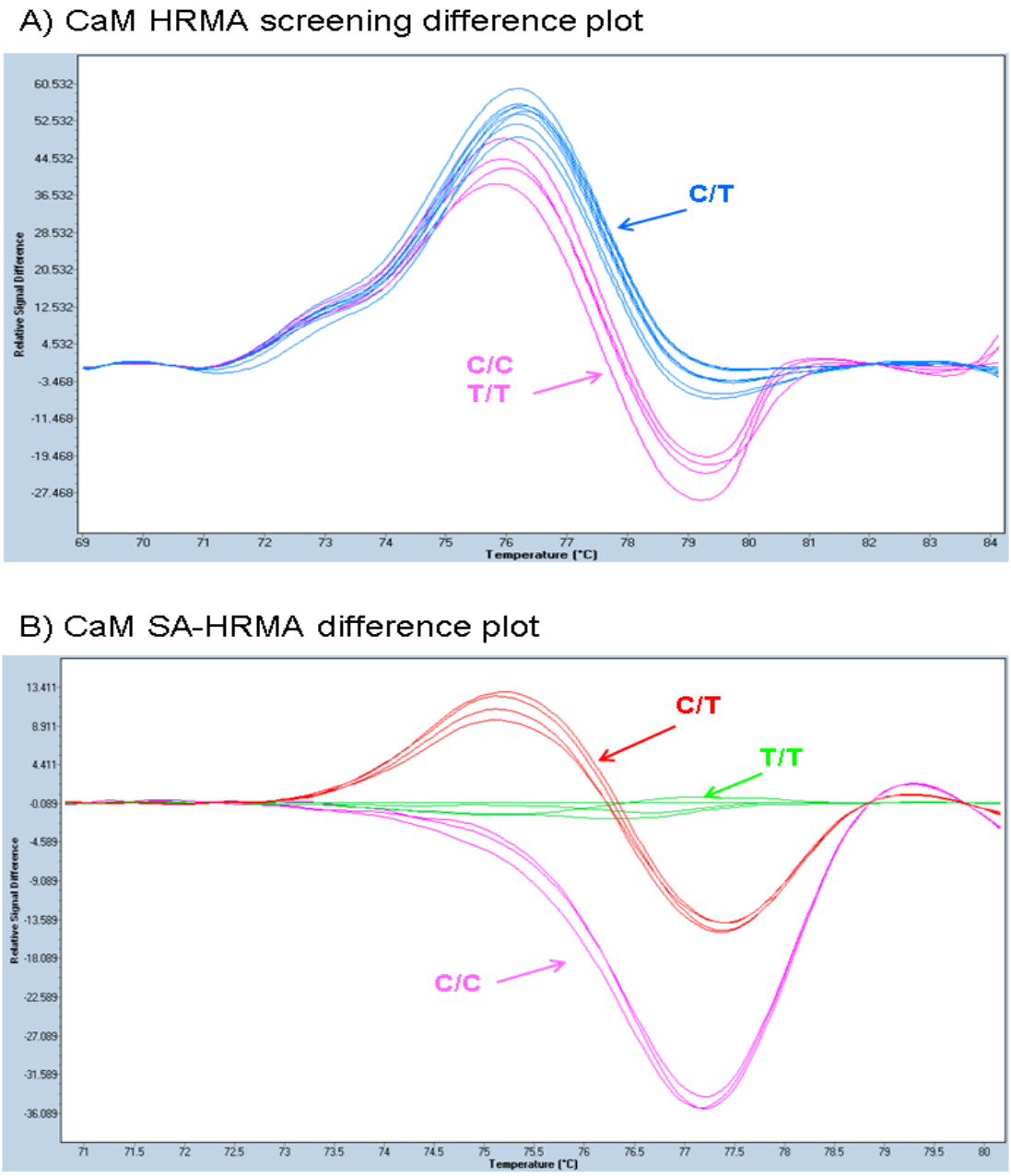

Figure 3. CaM HRMA of swordfish. (A) The initial HRMA SNP screening fluorescence difference plot of the calmodulin locus (504 bp) for a swordfish sample (n=11). The plot depicts two melting profile variants representing three homozygous C/C, four T/T (pink), and four heterozygous C/T (blue) individuals. Homozygous individuals of either allele cannot be differentiated from each other and resemble heterozygous melting profiles. (B) The SA-HRMA fluorescence difference plot of the calmodulin locus (49 bp) in multiple individuals using the homozygous T/T (green) as reference. The SA-HRMA easily distinguishes homozygous C/C (pink) and T/T and the heterozygous (red) individuals by the shape of the melting curves and ΔTm.

Evaluation of SA-HRMA genotyping

Based on the criteria of marker selection detailed above, two of the ten genes were excluded from further HRMA genotyping. In AldB an imperfect short tandem repeat (STR) was identified for microsatellite genotyping. In Act2A multiple base-pair neutral SNPs (i.e. two or more complementary SNPs) surrounded the SNP of interest. The SNP coincided with a Hpy8II restriction site polymorphism that was genotyped via agarose gel electrophoresis. Of the remaining eight loci, reliable SA-HRMA genotyping assays targeting a single SNP were developed for ARP, ATPsβ, CaM, and SRP54. SA-HRMA primers were designed to maximize the ΔT_m between alleles to decrease potential genotyping errors (Liew *et al.* 2004), and to increase amplification success (Figure 2). Resulting SA-HRMA amplicons ranged in size from 41-75 bp (**Table 3**). To reduce genotype masking, SA-HRMA primers flanked closely the targeted SNP.

HRMA has several advantages for population studies. Since it is a closed-tubed genotyping assay, cross-PCR contamination and genotyping errors associated with post-PCR sample manipulation are minimized. HRMA is also rapid and when coupled with real-time (RT) PCR platforms (e.g. LightCycler 480) the entire process, from PCR to genotyping, can be completed in less than an hour. The use of SA-HRMA has additional advantages over traditional HRMA. First, SA-HRMA is even more rapid as cycling times, temperature holds, and denaturation temperatures are substantially reduced with small amplicons. Second, targeting amplicons <60 bp reduces genotype masking. Third, decreased amplicon length increases the ΔT_m between genotypes thereby increasing genotyping accuracy (Gundry *et al.* 2008). Fourth, the only additional

expenditure associated with SA-HRMA, compared to other alternative RT-PCR SNP genotyping technologies, is a saturation dye (e.g. LCGreen+, EvaGreen, etc.) which represents a fraction of the cost. Altogether, SA-HRMA is a low cost genotyping alternative for population studies.

It is important to note that there are disadvantages associated with SA-HRMA. First, it is not always possible to design SA-HRMA primers flanking the SNP(s) of interest. The inability to identify suitable SA-HRMA primers (e.g. suboptimal primer sequence, additional SNPs in flanking regions, etc.) precluded genotyping Mlc2 using SA-HRMA. Second, when multiple SNPs are targeted, in particular those that are base-pair neutral, it may be difficult to differentiate among melting curves. When multiple SNPs (2-5) were targeted with SA-HRMA primers for GpHr, ldhA, and ANT, not all of the genotypes could be differentiated. Third, primer-dimer formation may obscure genotyping (Liew *et al.* 2004). Although primer dimers were the most common artifacts observed, interference with genotyping was not observed since the T_m of the targeted alleles were substantially higher.

UP-HRMA genotyping

For the candidate loci (ANT, Mlc2, GpHr, and ldhA) that were not amenable to SA-HRMA (i.e. poor primer placement, multiple SNPs, etc.), UP-HRMA genotyping assays were successfully developed. Asymmetric PCR chemistry and cycling parameters required additional optimization, as compared to SA-HRMA; however, UP-HRMA assay optimization was easily attained. The number of alleles genotyped via

UP-HRMA ranged from three to seven per locus with probe lengths ranging from 33-50 nucleotides (**Table 3**). UP-HRMA shares all of the advantages outlined for SA-HRMA (low cost, rapid, closed-tube, etc.). In addition, when compared to other SNP genotyping methods (i.e. TaqMan, molecular beacons, sequencing, RE digest, etc.), UP-HRMA has the ability to identify and differentiate multiple SNPs in a single closed-tube assay. The inclusion of multiple SNPs within-loci has been shown to increase the power to detect population differentiation (Morin *et al.* 2009). Studies that utilize sequence data or multiple SNPs within-loci rely on Bayesian methods for haplotype reconstruction (Stephens & Donnelly 2003). Such inference is not necessary for a validated UP-HRMA genotyping assay as haplotypes are differentiated from probe melting curves (**Figure 4**). Furthermore, UP-HRMA can reveal new SNPs while genotyping populations at a substantially reduced cost and in a fraction of the time compared to sequencing technologies. Of the 19 SNPs targeted by UP-HRMA, 36.8% were identified during UP-HRMA genotyping and not from preliminary screening. These additional 7 SNPs (ANT:3, Mlc2:1, GpHr:1, and ldhA:2) would not have been detected by non-sequencing genotyping assays. Alternative non-sequencing genotyping assays would potentially result in: 1) failed genotyping reactions or 2) genotyping masking errors (Cox & Kraft 2006).

Polymorphism evaluation of the eight HRMA markers in swordfish

Analysis of adult swordfish from the NA (n=475), MED (n=59), and SA (n=240) found no significant linkage disequilibrium between loci. Exact tests of Hardy-

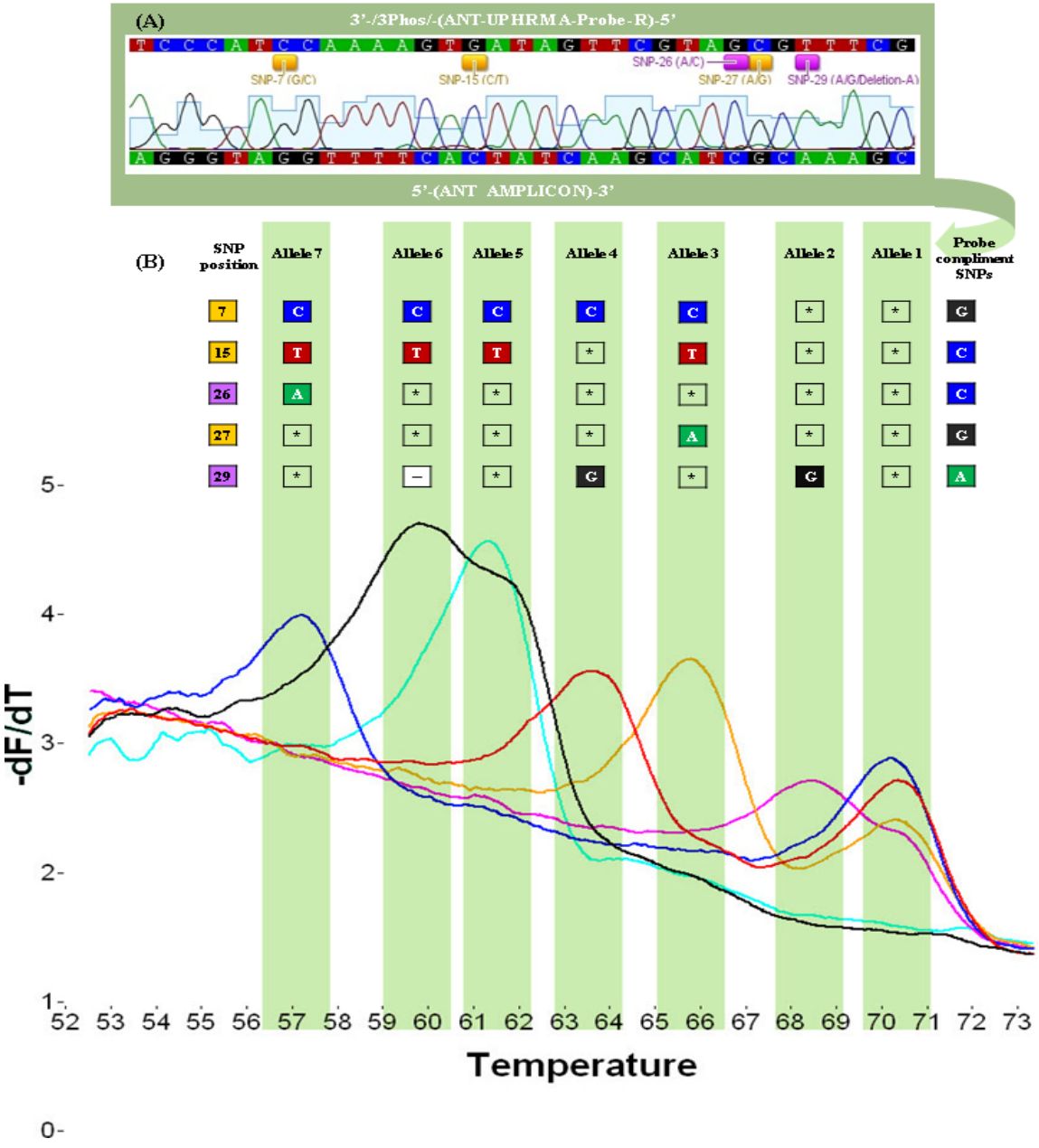

Figure 4. ANT UP-HRMA of swordfish. (A) Stretch of sequence of the ANT gene targeted with an unlabeled probe. SNPs identified via preliminary sequencing are identified with orange blocks and post UP-HRMA sequencing with magenta blocks. For each SNP, the relative nucleotide position and the alternative complement substitution to the probe (in parentheses) are given. The electropherogram of the corresponding sequence of a homozygous individual (allele 1) is depicted. (B) Probe complement SNPs (*) and corresponding mismatches for each SNP define the T_M and corresponding melting profiles of 6 genotypes that include alleles 1-7, as a function of fluorescence over temperature (°C).

Weinberg equilibrium (HWE) calculated using GENEPOP (Raymond & Rousset 1995b; Rousset 2008) found significant deviations from HWE (P<0.05) in CaM, ARP, and Mlc2 in the NA, as well as GpHR in both the NA and SA. Estimates for H_e varied between 0.281 – 0.532 (**Table 3**). Deviations from HWE were expected as many of the samples were collected in areas of mixing between stocks (Chapter IV). To assess whether deviations in HWE could be the result of genotyping error rather than population admixture adult swordfish sampled from breeding areas in NA (n=49), MED (n=59), and SA (n=42) were analyzed separately (**Table 3**). Since no deviations from HWE or heterozygous excess were observed in the analysis of adult swordfish in spawning grounds, genotyping errors in HRMA appear to be very low (Hosking *et al.* 2004; Wigginton *et al.* 2005). Low genotyping error is consistent with studies that have evaluated HRMA for clinical applications (Martino *et al.* 2010; Wittwer 2009). Furthermore, pairwise F_{ST} values (Weir & Cockerham 1984) among NA, SA, and MED samples using all the individuals (n=774) whose SNPs were genotyped with HRMA were up to one order of magnitude greater than the F_{ST} values reported using microsatellite data (Kotoulas *et al.* 2007) (**Table 4**). The levels of population differentiation in this study are congruent with previous mtDNA studies of population structure in Atlantic swordfish (summarized in Alvarado Bremer *et al.* 2007), further validating the effectiveness of HRMA genotyping in population genetic studies.

Table 4. A comparison of pairwise FST values calculated from SNPs contained in eight nuclear loci (below the diagonal) for North Atlantic (NA; n=419), Mediterranean (MED; n=59), and South Atlantic (SA; n=296) populations in this study and the F_{ST} values calculated from four microsatellites reported by Kotoulas et al. 2007 (above diagonal). All values are significantly different from zero (P<0.001).

	NA	MED	SA
NA	-	0.02981	0.00112
Med	0.03994	-	0.03340
SA	0.03934	0.12615	-

Cross-species applications

Due to the potential high cost and time investment associated with SNP discovery and genotype assay design in non-model species, cross-species utility is desirable. However, the applicability of SNP genotyping assays in a cross-species is decreased by 1) amplification failures (i.e. primer mismatch); 2) absence of SNPs within the targeted segment; or 3) neighboring SNPs interfering with assay probes in the non-target species. Miller et al. (2011) evaluated the utility of the OvineSNP50 BeadChip, designed for domestic sheep, to score SNPs in other ungulates. Cross-species amplification success for 49,034 loci was ~98%; however, the utility of the assay to screen variation was extremely low with only 1% of the loci containing polymorphisms. This strongly indicates that the major source of failure in cross-species application is not amplification failures, but the absence of SNPs within the targeted loci and or genotype masking.

Studies have found introns to contain two to five times more SNPs than exons (Brumfield *et al.* 2003). By designing UP-HRMA primers in highly conserved exons, and probes in highly polymorphic introns, amplification success is maintained while maximizing the potential presence of SNPs within targeted regions in cross-species. Whereas, SA-HRMA requires non-degenerative forward and reverse primers to differentiate homoduplex from heteroduplex curves, UP-HRMA can utilize universal primers which may include mismatches. Since we found 2.39×10^{-2} SNPs per intron base and UP-HRMA probes length averaging ~40+ nucleotides, the probability of identifying an additional SNP even when the targeted SNP is absent in the non-target species is high with UP-HRMA. Since swordfish are a monotypic species that diverged from Istiophoridae over 50 Ma (Fierstine & Applegate 1974), cross species amplifications using the swordfish specific HRMA primers are not expected to be successful. However, an examination (unpublished Lu *et. al.*) of zinc finger protein sequences amplified with the universal ZnF-GW primers (Peichel *et al.* 2004) in sailfish (*Istiophorus platypterus*), blue marlin (*Makaira nigricans*), and Atlantic white marlin (*Kajikia albida*) individuals helps illustrate the cross species utility of UP-HRMA (**Figure 5**). By comparison, cross-species amplification using site specific SNP technologies (i.e. TaqMan, molecular beacons, etc.) would be either uninformative or result in potential genotyping error as neighboring SNPs are present in the locus (**Figure 5**). In addition, while site-specific SNP technologies would identify only two alleles, a total of six alleles could be identified using UP-HRMA from the specific melting curves produced by three adjacent SNPs without need for Bayesian haplotype reconstruction.

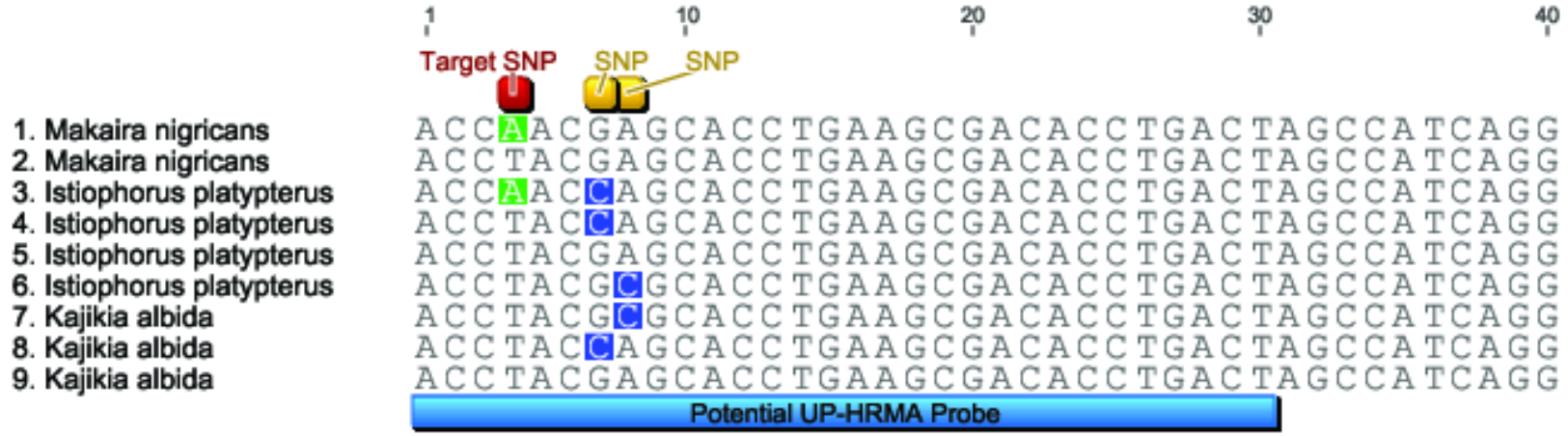

Figure 5 An alignment of a segment of the zinc finger protein (ZnF) gene of blue marlin (*Makaira nigricans*), sailfish (*Istiphorus platypterus*), and Atlantic white marlin (*Kajikia albida*). The SNP (red) target in *M. nigricans* is also polymorphic in *I. platypterus*; however, additional adjacent SNPs (yellow) in *I. platypterus* may result in genotyping error when using site specific SNP technologies (i.e. TaqMan, molecular beacons, etc.). In *K. albida* the targeted SNP (red) is not polymorphic therefore cross species amplification would be uninformative. By contrast, cross species amplification using UP-HRMA (probed segment in blue at bottom) would preclude genotyping error and the additional adjacent SNPs (yellow) in both the *I. platypterus* and *K. albida* probed segment could potentially be scored as well.

As shown in this study, neighboring SNPs do not cause genotype masking in UP-HRMA.

Conclusions

I found HRMA to be a highly sensitive, closed-tube, low cost, amenable to high through-put (n=774) alternative for initial SNP screening and subsequent genotyping in non-model population studies. Both SA-HRMA and UP-HRMA genotyping assays scored the alleles of single and multiple SNPs reliably such that haplotype reconstruction is not necessary. IWe outline a workflow for HRMA SNP discovery and genotyping that could serve as a template for other non-model population genetic studies.

CHAPTER IV

GENETIC POPULATION STRUCTURE AND ADMIXTURE OF ATLANTIC SWORDFISH (*XIPHIAS GLADIUS* L.)

Introduction

Due to their high migratory potential, pelagic fishes are not expected to display genetic structure both within oceans and in some cases also between oceans (Avise 2000; Graves 1998). The absence of obvious oceanographic barriers to gene flow, together with adaptations that facilitate long distance dispersal of pelagic eggs and larvae, active movement of juveniles and adults, high fecundity, eurythermal physiologies, and opportunistic feeding behaviors, all contribute to the observed widespread distribution of many cosmopolitan pelagic fishes, such as tunas, billfishes, dolphinfishes, and lamnid sharks., Conventional tagging studies have confirmed the expectation of high vagility and genetic analyses have been consistent with panmixia. For instance, common dolphinfish (*Coryphaena hippurus*), wahoo (*Acanthocybium solandri*), and skipjack tuna (*Katsuwonus pelamis*) lack genetic population structure among the world's oceans (Díaz-Jaimes *et al.* 2006; Ely *et al.* 2005; Theisen *et al.* 2008). Similarly, genetic investigations in several species of Atlantic fishes have failed to find significant population substructure within this basin, including shortfin mako (*Isurus oxyrinchus*) (Schrey & Heist 2003), blue marlin (*Makaira nigricans*) and sailfish (*Istiophorus platypterus*) (Graves & McDowell 2003), bigeye tuna (*Thunnus obesus*)

(Gonzalez et al. 2008), and yellowfin tuna (*Thunnus albacares*) and skipjack tuna (Ely *et al.* 2005). By contrast, in swordfish (*Xiphias gladius* L.) significant genetic differentiation has been documented within the Atlantic Ocean (Alvarado Bremer *et al.* 2005a; Alvarado Bremer *et al.* 1996; Chow & Takeyama 2000) and also within the Mediterranean Sea (Viñas *et al.* 2010).

Swordfish is large epipelagic monotypic species with cosmopolitan distribution, and this species is capable of conducting long distance movements. Swordfish are subject to intensive commercial exploitation worldwide, and in the Atlantic Ocean they are managed by the International Commission for the Conservation of Tunas (ICCAT) as two separate stocks: the North Atlantic and the South Atlantic separated at 5°N. North Atlantic swordfish in turn is managed separately from Mediterranean swordfish with a boundary at the Strait of Gibraltar. It should be noted that previous genetic work (Chow *et al.* 2007; Viñas *et al.* 2007) has provided evidence that suggest mixing areas beyond current management boundaries.

Previous genetic studies on Atlantic swordfish have relied on mitochondrial DNA (mtDNA) (see Alvarado Bremer *et al.* 2007 for summary), single copy nuclear DNA (scnDNA) (Chow *et al.* 2007; Chow & Takeyama 2000; Grieg *et al.* 2000), and microsatellite loci (Kasapidis *et al.* 2007; Kasapidis *et al.* 2008; Kotoulas *et al.* 2007). Analyses with all three types of marker are concordant regarding the significant differentiation between Mediterranean and Atlantic swordfish. In addition, nucleotide sequence data (Alvarado Bremer *et al.* 1995; Alvarado Bremer *et al.* 2005a) and PCR-RFLP (Alvarado Bremer *et al.* 1996; Chow *et al.* 1997) of the mtDNA control region I

(CR-I) revealed significant heterogeneity of lineages of two highly divergent clades that genetically differentiate Northwest and South Atlantic swordfish. Single nuclear locus studies analyzing variation contained in introns have provided concordant with mtDNA data regarding the difference between the Northwest and South Atlantic (Chow *et al.* 2007; Chow & Takeyama 2000; Grieg *et al.* 1999). In spite of the agreement between mtDNA and nDNA data, there are limitations in these previous studies. First, sampling coverage has been limited and estimates of admixture as well as a clarification of relationship of Northeast Atlantic swordfish with the Northwest and South Atlantic populations was not possible with these data.

By contrast, multilocus analyses of microsatellite data (Kasapidis *et al.* 2007; Kotoulas *et al.* 2007) and Bayesian individual assignment had the potential to identify zones of admixture, but failed to distinguish among North Atlantic and South Atlantic swordfish. Previously microsatellite loci were utilized almost exclusively in Bayesian analyses of population admixture; however there is an increasing adoption of single nucleotide polymorphisms (SNPs) in such studies. The application of SNPs have revealed lower associated genotyping errors, simpler mutation models, comparable differentiation power, higher amenability to automation and high through-put technologies, and increased amplification success with low-quality and ancient DNA as compared to microsatellites (Morin *et al.* 2004; Morin *et al.* 2009; Ryman *et al.* 2006). Since levels of genetic differentiation among Atlantic swordfish associated with SNPs, as compared to microsatellites, is an entire order of magnitude greater (see Chapter III),

Bayesian individual assignment analysis using SNPs may be able to provide the estimates of admixture currently lacking in Atlantic swordfish.

In this paper we provide molecular evidence of the genetic differentiation between North Atlantic, South Atlantic, and Mediterranean swordfish based on the characterization of single nucleotide polymorphisms (SNPs) contained in multiple nuclear loci. Bayesian individual assignment is used to evaluate the extent of population admixture and we discuss potential mechanisms of isolation.

Materials and methods

Sampling

A total of 774 swordfish specimens from 18 localities in the Atlantic and Mediterranean were collected from 1991-2006 (**Table 5**). Tissue samples were collected by observers on commercial longline vessels or obtained from collaborating researchers. In both instances location of capture for individual fish were recorded. Larval swordfish (n=52), collected as part of icthyoplankton surveys in the northern Gulf of Mexico during the summers of 2005-2006 (Rooker *et al.* 2012), were also included in the analyses. The management boundaries of 5°N and the Strait of Gibraltar (ICCAT) were used for assignment of localities to the North Atlantic, South Atlantic, or Mediterranean populations. Tissue was either frozen or stored in 70 % ethanol before shipment to the laboratory.

Table 5. Sampling localities for 774 swordfish subdivided into three stocks (North Atlantic, Mediterranean, South Atlantic) by ICCAT management boundaries of 5°N and Strait of Gibraltar

Population	Sample Locality	Latitude	Longitude	Sampling Date(s)	n
North Atlantic					
1	Gulf of Mexico	26-28N	86-93W	06/06-07/06, 06/07	52
2	Florida	21-32N	78-85W	1/93-8/93	49
3	Northeast US	35-42N	67-74W	8/90-11/90, 1/91-3/91	42
4	Antillies	12-20N	49-64W	10/92-2/93, 2/94, 1/95	48
5	east of Flemish Cap	43-47N	37-41W	9/04-11/04	17
6	CentralNorth Atlantic	32-47N	37-43W	11/91, 12/95, 9/04-11/04	20
7	Iberian	35N	10-20W	11/91, 12/92, 3/93-3/93, 12/95-4/96	51
8	Strait of Gibraltar	33-35N	5-10W	8/92, 6/93-9/93, 5/96-11/96	83
10	Morocco	30-33N	12-15W	4/93-5/93, 10/96-11/96, 6/02-8/02	20
11	Western Sahara	20-28N	12-20W	2/92, 5/92, 6/93, 12/95, 4/96, 7/96-11/96	37
12	Cape Verde	5N-17N	21-32W	9/05-10/05, 8/04-12/04	56
Mediterranean					
9	Mediterranean	35-39N	0-8E	5/92-7/92, 8/03-12/03	59
South Atlantic					
13	Equatorial Brazil	1S-3N	23-35W	11/91, 3/00, 9/04	42
14	Gulf of Guinea	5N	4W	11/91, 7/98-8/98	45
15	Central South Atlantic	14-16S	10-17W	10/04-12/04	28
16	Brazil	15-23S	22-27W	3/96, 5/96, 4/01, 12/04	35
17	Brazil-Uruguay	27-37S	32-42W	8/95-10/95, 4/01, 6/03-7/03	44
18	Namibia	26-27S	10-11E	7/99-8/99	46

DNA extraction and nuclear loci genotyping

Genomic DNA was isolated from tissue with a modified TENS and Proteinase K [20mg/μL] digestion followed by ethanol precipitation without organic extraction (Grieg 2000). Ten nuclear loci were amplified and genotyped for genetic population structure analyses: acidic ribosomal phosphoprotein P0 (ARP); adenine nucleotide translocator (ANT); aldolase-B (AldB); alpha-skeletal actin (Act2α); ATP synthase beta-subunit (ATPsβ); Calmodulin (C*aM*); Golgi pH regulator (GpHr); lactose dehydrogenase A (ldhA); myosin light chain (Mlc2); and signal recognition particle 54 (SRP54). Procedures for the amplification and subsequent genotyping of single nucleotide polymorphisms (SNPs) for short amplicon high-resolution melting analysis (SA-HRMA) of ARP, ATPsβ, C*aM*, and SRP54 and unlabeled probe high-resolution melting analysis (UP-HRMA) of ANT, GpHR, ldhA, and Mlc2 are described in Smith et al. (in review). Two loci, AldB and Act2α, were scored as size polymorphisms through agarose gels and capillary electrophoresis as follows.

A simple sequence repeat (SSR) was identified in AldB (see Grieg 2000 and Chapter III) and the following primers were used for amplification; 5'-VIC-TGTGCCCAGTATAAGAAGGATGG-3' and 5'-CTGTGGAGAATCAGGGCTCC-3' (JX042447). Polymerase chain reactions (PCR) were conducted in 12.5 μL reactions containing 10 ng of genomic DNA, 1X Econotaq Green Master Mix (Lucigen), and 0.20 μM of each primer. Thermocycling was performed on an Eppendorf Mastercycler with an initial denaturation of 10 min at 95 °C followed by 35 cycles denaturing for 1 min at 94 °C, annealing for 1 min at 54 °C, and extension for 1 min at 72 °C. PCR products

were diluted 1:10 and 1 μL of PCR template, 0.2 μL of ROX size standard (Applied Biosystems), and 10 μL of Hi-Di formamide were run on an ABI 3130 Genetic Analyzer (Applied Biosystems). Microsatellites were analyzed using GeneMapper v.4.0 (Applied Biosystems) and scored by size.

In Act2α a SNP that corresponded to a Hpy8I restriction enzyme site was genotyped as follows. PCR of Act2α followed the same chemistry and thermo-cycling procedures as AldB with the following primers; 5'-GTCACCGGAGTCCAGGACG-3' and 5'-ATCTGGCACCACACCTTCTACAA-3' (JX042448). PCR products were visualized in a 1% agarose gel via gel electrophoresis to verify amplification. Restriction enzyme digests were conducted in 10 μL volumes containing 2-4 μL PCR product, 1X Buffer TANGO (Fermentas), and 0.4 U of Hpy8I (Fermentas) and were digested for 16 hours at 37°C and scored in 2% TA agarose gels run at 100V for 30 min. RFLP's were visualized on a Gel Doc XR (Bio-Rad) UV transilluminator and images were captured and scored in Quality One v.4.6 (Bio-Rad).

Data analysis

Genetic diversity. The number of alleles, observed (H_o) and expected (H_e) heterozygosities under Hardy-Weinberg equilibrium (HWE), and inbreeding coefficients (F_{IS}) were calculated using GENALEX version 6.41 (Peakall & Smouse 2006). Departure from HWE using an exact probability test *(Fisher 1935; Guo & Thompson 1992)* and global linkage disequilibrium using a maximum likelihood-ratio test (Raymond & Rousset 1995a) were both computed in GENPOP version 4.1 (Markov

chain parameters: 10000 dememorization steps, 1000 batches, 10000 iterations per batch) (Raymond & Rousset 1995b; Rousset 2008). Sequential Bonferroni corrections were applied to adjust the levels of statistical significance for multiple comparisons (Rice 1989). An F_{ST}-outlier detection method, in which observed locus F_{ST} values are compared to calculated global F_{ST} values expected under neutrality using coalescent simulations (Beaumont & Nichols 1996) was performed with the program LOSITAN (Antao *et al.* 2008). Putative loci under selection were detected using the default settings, 50,000 permutations, and with a 99% confidence interval. The assignment power of each locus was ranked *a posteriori* to Bayesian analysis using the critical population method in WHICHLOCI v1.0 (Banks *et al.* 2003) using samples from localities identified as non-mixing areas.

Population differentiation. A hierarchical analysis of molecular variance (AMOVA)(Excoffier *et al.* 1992) was implemented in Arlequin version 3.5 (Excoffier *et al.* 2005) to estimate levels of population subdivision and population differentiation was evaluated using global and pairwise F_{ST} tests (Weir & Cockerham 1984). Using AMOVA groupings of two (MED and Atlantic) and three (MED, NA, and SA) that adhered to the current management regions were initially evaluated. Alternative groupings were subsequently evaluated a posteriori to F_{ST} and Bayesian analysis. Slatkin's linearized F_{ST} (Slatkin 1993) was also calculated in Arlequin. Principle coordinates analysis (PCoA) (Orlóci 1978) of the standardized covariance of population

pairwise genetic distance matrix was completed in GenAlEx version 6.4 (Peakall & Smouse 2006).

Genetic clustering analysis. Genetic population structure and patterns of inter-population gene flow were assessed by Bayesian inference implemented in STRUCTURE version 2.3 (Pritchard *et al.* 2000). Previous genetic studies using mtDNA and a single nuclear locus identified comparatively low levels of gene flow in between North and South Atlantic swordfish (Alvarado Bremer *et al.* 2005a; Chow *et al.* 2007; Chow *et al.* 1997). Accordingly, a no admixture ancestry with correlated allele frequencies model, as outlined in Falush et al. (2003) was adopted. Compared to freshwater and anadromous fishes, marine fishes display weak levels of population structure ($F_{ST} < 0.20$) (Waples 1998) therefore, the LOCPRIOR option was implemented to infer weak population structure using an a priori group sampling as outlined in Hubisz et al. (2009). The number of clusters (K) was estimated using an *ad hoc* approach (Pritchard *et al.* 2000) by obtaining the mean posterior probability of the data (L(K)) and the ΔK approach of Evanno et al. (2005) using STRUCTURE HARVESTER v0.6.92 (Earl & Vonholdt 2012). Twenty independent runs for each K value (1-10) were performed using 100,000 Markov chain Monte Carlo (MCMC) iterations with a burn-in period of 100,000. Results from STRUCTURE were compared with the results from GENELAND (Guillot *et al.* 2005), which incorporates individual spatial and genetic data to infer population structure and spatial boundaries between clusters. Individual spatial coordinates were set to the corresponding latitude and longitude of sampling. An

uncertainty value on the spatial coordinates of each individual sample was set to 30 decimal degrees which corresponds to the patterns of migration suggested by tagging of North Atlantic swordfish (Neilson *et al.* 2009; Sedberry & Loefer 2001). Correlated allele and spatial models were implemented for twenty independent runs with 100,000 MCMC iterations and a burn-in of 10,000 iterations for *K* (1-8) values. After *K* was estimated, 25 independent runs at the estimated *K* value were run in GENELAND, 50 independent runs in STRUCTURE, and the optimal alignment of replicates was completed using CLUMPP version 1.1.12 (Jakobsson & Rosenberg 2007).

Results

Hardy-Weinberg, linkage disequilibrium, and power assignment

A total of 774 swordfish were genotyped successfully at all ten loci. The number of alleles per locus ranged from 2 to 6. Fisher's exact probability tests for departure from HWE were not significant ($P > 0.05$) after Bonferroni correction (**Table 6**). Genotypic linkage was not significant among the 44 possible pairs of nuclear loci across all samples with ($P > 0.01$) and without ($P > 0.05$) Bonferroni correction. Exact tests for genotypic linkage disequilibrium within each sample revealed no significant *P* values after Bonferroni correction ($P > 0.05$). Initial F_{ST} outlier analysis of the ten loci of all individuals from the 18 localities identified C*aM* as a significant outlier ($P < 0.01$) (**Figure 6A**). However, restricting the analysis samples to corresponding spawning areas in the Northwest Atlantic, South Atlantic, and Mediterranean yielded no loci as

Table 6. Genetic diversity of 10 nuclear loci within 18 swordfish populations.

		Locus									
Population		ARP	ALDB	GpHR	Act2α	ATPsβ	CaM	Mlc2	SRP54	LDHA	ANT
1	*A*	2	4	5	2	2	2	3	2	3	5
	H_o	0.288	0.288	0.615	0.231	0.500	0.346	0.173	0.327	0.385	0.442
	H_E	0.447	0.287	0.581	0.473	0.453	0.497	0.206	0.322	0.450	0.567
	F_{IS}	0.354	-0.005	-0.059	0.513	-0.105	0.304	0.161	-0.014	0.145	0.220
2	*A*	2	4	5	2	2	2	3	2	3	5
	H_o	0.347	0.224	0.571	0.592	0.469	0.510	0.265	0.388	0.633	0.571
	H_E	0.479	0.205	0.512	0.500	0.493	0.497	0.320	0.359	0.468	0.522
	F_{IS}	0.276	-0.097	-0.115	-0.184	0.047	-0.027	0.170	-0.079	-0.352	-0.094
3	*A*	2	4	5	2	2	2	3	2	3	5
	Ho	0.381	0.238	0.571	0.429	0.429	0.476	0.357	0.310	0.476	0.405
	HE	0.472	0.218	0.521	0.459	0.427	0.499	0.381	0.262	0.447	0.504
	FIS	0.192	-0.094	-0.097	0.067	-0.003	0.045	0.062	-0.183	-0.065	0.197
4	*A*	2	4	5	2	2	2	3	2	3	6
	H_o	0.417	0.188	0.521	0.458	0.458	0.500	0.458	0.292	0.583	0.542
	H_E	0.444	0.209	0.513	0.413	0.457	0.499	0.400	0.330	0.476	0.545
	F_{IS}	0.062	0.101	-0.015	-0.109	-0.002	-0.002	-0.147	0.116	-0.225	0.007
5	*A*	2	3	4	2	2	2	2	2	2	5
	H_o	0.471	0.176	0.353	0.353	0.412	0.471	0.353	0.235	0.588	0.412
	H_E	0.415	0.164	0.424	0.415	0.389	0.498	0.291	0.291	0.457	0.356
	F_{IS}	-0.133	-0.074	0.167	0.150	-0.058	0.056	-0.214	0.190	-0.288	-0.155
6	*A*	2	3	3	2	2	2	2	2	3	4
	H_o	0.350	0.150	0.300	0.200	0.350	0.400	0.350	0.250	0.700	0.500
	H_E	0.489	0.141	0.339	0.255	0.349	0.480	0.349	0.219	0.540	0.529
	F_{IS}	0.284	-0.062	0.114	0.216	-0.004	0.167	-0.004	-0.143	-0.296	0.054
7	*A*	2	3	3	2	2	2	2	2	3	4
	H_o	0.350	0.150	0.300	0.200	0.350	0.400	0.350	0.250	0.700	0.500
	H_E	0.489	0.141	0.339	0.255	0.349	0.480	0.349	0.219	0.540	0.529
	F_{IS}	0.284	-0.062	0.114	0.216	-0.004	0.167	-0.004	-0.143	-0.296	0.054
8	*A*	2	4	5	2	2	2	3	2	3	5
	H_o	0.301	0.301	0.590	0.349	0.422	0.470	0.494	0.108	0.494	0.518
	H_E	0.384	0.294	0.503	0.319	0.451	0.497	0.506	0.164	0.511	0.532
	F_{IS}	0.215	-0.024	-0.173	-0.097	0.065	0.055	0.023	0.340	0.034	0.026
9	*A*	2	2	4	2	2	2	2	2	3	3
	H_o	0.339	0.390	0.525	0.356	0.475	0.424	0.559	0.051	0.424	0.627
	H_E	0.324	0.403	0.505	0.314	0.493	0.479	0.500	0.050	0.514	0.539
	F_{IS}	-0.046	0.032	-0.041	-0.134	0.037	0.116	-0.119	-0.026	0.176	-0.163
10	*A*	2	4	5	2	2	2	3	2	3	4
	H_o	0.400	0.200	0.400	0.300	0.600	0.350	0.200	0.200	0.400	0.400
	H_E	0.480	0.186	0.505	0.375	0.495	0.439	0.395	0.180	0.499	0.468
	F_{IS}	0.167	-0.074	0.208	0.200	-0.212	0.202	0.494	-0.111	0.198	0.144
11	*A*	2	4	5	2	2	2	3	2	3	6
	H_o	0.432	0.108	0.568	0.216	0.541	0.459	0.297	0.297	0.486	0.622
	H_E	0.456	0.104	0.581	0.307	0.482	0.407	0.360	0.290	0.481	0.555
	F_{IS}	0.051	-0.039	0.024	0.295	-0.121	-0.130	0.174	-0.026	-0.011	-0.120
12	*A*	2	6	5	2	2	2	3	2	3	6
	H_o	0.536	0.232	0.500	0.554	0.375	0.232	0.411	0.268	0.536	0.464
	H_E	0.497	0.213	0.464	0.481	0.500	0.205	0.406	0.305	0.526	0.439
	F_{IS}	-0.077	-0.090	-0.078	-0.152	0.250	-0.131	-0.012	0.121	-0.018	-0.057
13	*A*	2	3	5	2	2	2	3	2	3	5
	H_o	0.405	0.262	0.524	0.452	0.429	0.190	0.286	0.452	0.429	0.476
	H_E	0.477	0.234	0.535	0.350	0.459	0.172	0.338	0.350	0.489	0.458
	F_{IS}	0.152	-0.117	0.021	-0.292	0.067	-0.105	0.154	-0.292	0.123	-0.040
14	*A*	2	5	5	2	2	2	3	2	3	6
	H_o	0.511	0.222	0.556	0.422	0.489	0.156	0.400	0.333	0.533	0.600
	H_E	0.475	0.206	0.573	0.437	0.429	0.143	0.336	0.278	0.529	0.552
	F_{IS}	-0.075	-0.080	0.030	0.033	-0.141	-0.084	-0.190	-0.200	-0.009	-0.088
15	*A*	2	3	5	2	2	2	2	2	3	5
	H_o	0.571	0.393	0.393	0.286	0.643	0.179	0.250	0.357	0.571	0.321
	H_E	0.500	0.327	0.596	0.337	0.459	0.163	0.270	0.337	0.548	0.524
	F_{IS}	-0.143	-0.201	0.341	0.152	-0.400	-0.098	0.073	-0.061	-0.043	0.387
16	*A*	2	3	5	2	2	2	3	2	3	4
	H_o	0.514	0.257	0.429	0.486	0.543	0.200	0.571	0.457	0.629	0.514
	H_E	0.467	0.232	0.487	0.420	0.441	0.180	0.454	0.382	0.551	0.449
	F_{IS}	-0.101	-0.107	0.121	-0.156	-0.230	-0.111	-0.258	-0.197	-0.140	-0.144
17	*A*	2	6	5	2	2	2	3	2	3	4
	H_o	0.409	0.364	0.545	0.318	0.432	0.114	0.341	0.386	0.500	0.568
	H_E	0.474	0.339	0.549	0.397	0.456	0.146	0.334	0.407	0.468	0.493
	F_{IS}	0.137	-0.073	0.006	0.198	0.054	0.224	-0.022	0.050	-0.069	-0.154
18	*A*	2	4	5	2	2	2	3	2	3	5
	H_o	0.457	0.152	0.457	0.304	0.435	0.217	0.326	0.348	0.565	0.543
	H_E	0.471	0.182	0.473	0.364	0.440	0.194	0.368	0.315	0.512	0.534
	F_{IS}	0.032	0.165	0.034	0.164	0.011	-0.122	0.115	-0.105	-0.104	-0.018

A : The number of alleles; H_o : observed heterozygosity; H_E : expected heterozygositiy; F_{IS} : inbreeding cooeffcient

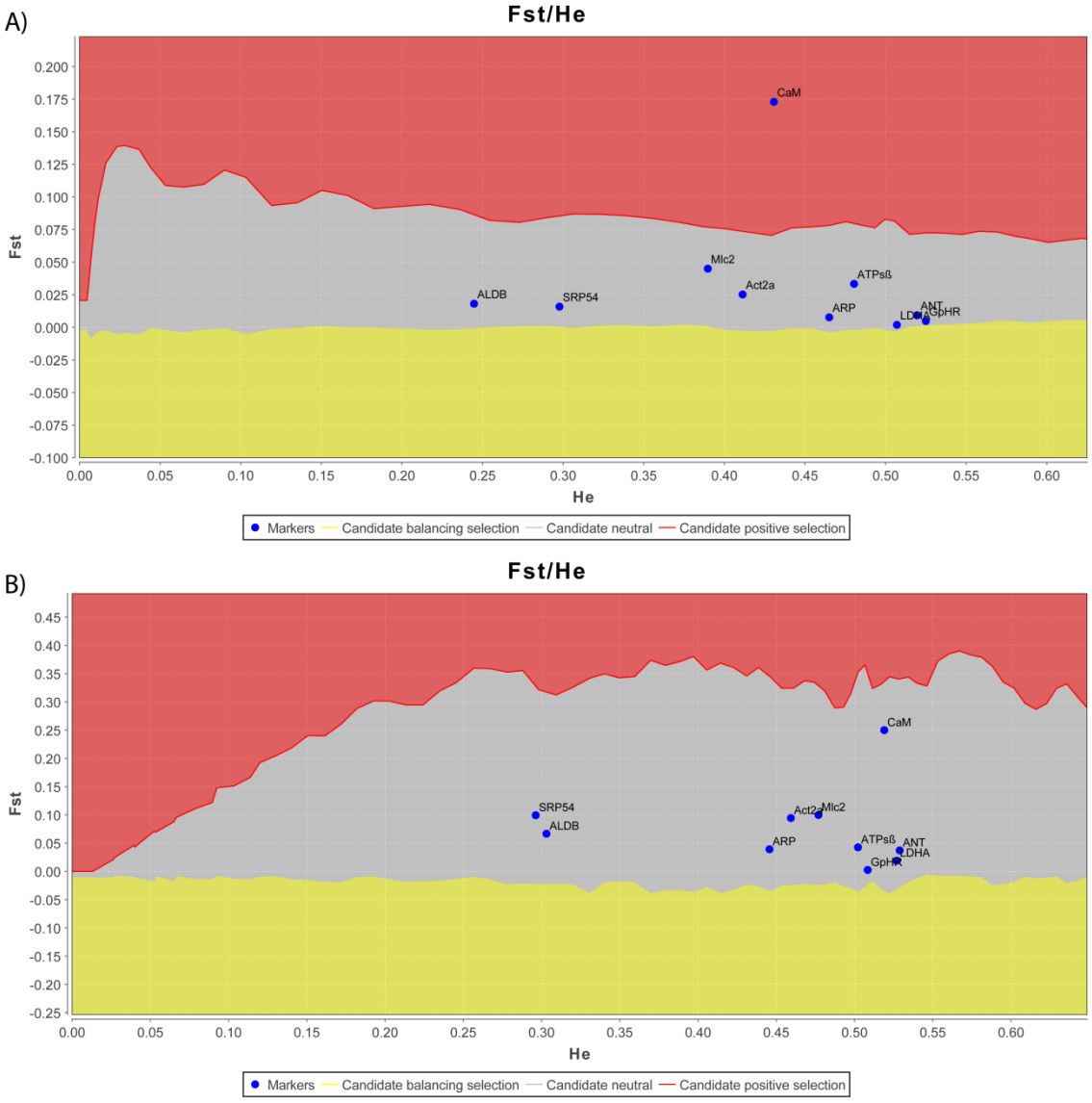

Figure 6. **Identification of candidate loci under selection inferred from F_{ST} outlier analysis (Antao *et al.* 2008; Beaumont & Nichols 1996) of ten nuclear markers using the individuals (A) from all 18 localities and (B) only the localities in known spawning areas where F_{ST} values of all the loci were notably greater and H_e for Act2α, AldB, ATPsβ, CaM, and Mlc2 increased as compared to (A).**

Table 7. Population pairwise F_{ST} of swordfish from 18 populations on the lower diagonal and P-values on the upper diagonal.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
1		0.401	0.319	0.438	0.272	*	0.161	***	***	0.112	**	***	***	***	***	***	***	***
2	0.000		0.108	0.106	*	**	0.190	***	***	0.118	**	**	***	***	***	***	***	***
3	0.001	0.006		0.470	0.419	0.226	0.074	***	***	0.393	*	***	***	***	***	**	***	***
4	0.000	0.006	0.000		0.429	0.250	0.380	***	***	0.385	0.055	***	***	***	***	**	***	***
5	0.003	**0.018**	0.000	0.000		0.411	0.188	***	***	0.410	0.089	**	*	***	**	*	**	*
6	**0.018**	**0.033**	0.006	0.004	0.000		0.078	***	***	0.428	0.325	**	*	**	*	*	**	0.080
7	0.005	0.003	0.007	0.000	0.007	0.013		***	***	0.423	0.279	***	**	**	**	*	**	**
8	**0.066**	**0.063**	**0.064**	**0.042**	**0.074**	**0.070**	**0.038**		0.158	**	***	***	***	***	***	***	***	***
9	**0.069**	**0.077**	**0.074**	**0.049**	**0.085**	**0.076**	**0.050**	0.003		***	***	***	***	***	***	***	***	***
10	0.009	0.010	0.001	0.001	0.001	0.000	0.000	**0.036**	**0.052**		0.421	0.226	0.318	0.138	0.224	0.179	0.090	0.411
11	**0.019**	**0.025**	**0.014**	0.010	0.015	0.003	0.002	**0.054**	**0.069**	0.000		**	0.203	0.084	0.104	0.063	0.103	0.291
12	**0.053**	**0.031**	**0.037**	**0.045**	**0.042**	**0.044**	**0.019**	**0.086**	**0.114**	0.005	**0.021**		0.079	*	0.064	0.134	**	*
13	**0.047**	**0.045**	**0.035**	**0.039**	**0.031**	**0.023**	**0.020**	**0.097**	**0.117**	0.002	0.004	0.008		0.417	0.443	0.420	0.415	0.412
14	**0.044**	**0.040**	**0.036**	**0.040**	**0.039**	**0.026**	**0.023**	**0.105**	**0.121**	0.008	0.009	0.011	0.000		0.382	0.409	0.394	0.421
15	**0.049**	**0.049**	**0.043**	**0.044**	**0.043**	**0.023**	**0.025**	**0.100**	**0.117**	0.005	0.009	**0.010**	0.000	0.000		0.301	0.413	0.429
16	**0.050**	**0.040**	**0.032**	**0.035**	**0.031**	**0.025**	**0.018**	**0.085**	**0.106**	0.007	0.012	0.005	0.000	0.000	0.002		0.405	0.412
17	**0.045**	**0.039**	**0.041**	**0.041**	**0.045**	**0.031**	**0.022**	**0.100**	**0.117**	0.011	0.008	**0.015**	0.000	0.000	0.000	0.000		0.399
18	**0.044**	**0.042**	**0.034**	**0.034**	**0.033**	0.014	**0.017**	**0.089**	**0.106**	0.001	0.002	**0.012**	0.000	0.000	0.000	0.000	0.000	

Significant Fst in bold, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

significant outliers ($P > 0.01$) and the F_{ST} values for all loci were higher as well as the H_e for Act2α, AldB, ATPsβ, CaM, and Mlc2. (**Figure 6B**). The power assignment of each loci using WHICHLOCI v1.0 identified CaM as the locus with the highest power of discrimination between North and South Atlantic populations followed by Mlc2, SRP54, AldB, ARP, Act2α, ldhA, GpHR, ATPsβ, and ANT respectively (see Appendix 21). However, the highest power of discrimination between the North Atlantic and Mediterranean populations was Mlc2, ATPsβ, Act2α, ldhA, ANT, AldB, SRP54, ARP, GpHR, and with CaM displaying the lowest power of discrimination (see Appendix 20)

Population differentiation

Pairwise F_{ST}. Multilocus pairwise F_{ST} values among the 18 populations ranged from less than 0.001 to 0.121 (**Table 7**). The largest level of differentiation was between Mediterranean and South Atlantic populations ($F_{ST>}0.081 – 0.121$). Levels of gene flow (*Nm*) were estimated among the pooled NA (1-5), MED (8-9), and SA (12-18) populations without samples identified as admixture zones (6-7, 11-10) were less than nine migrants per generation (**Table 8**). Slatkin's linearized F_{ST} values (**Figure 7**) highlights the homogeneity between the Mediterranean (8) and the adjacent area west of the Strait of Gibraltar (9) and the high degree of differentiation of these two areas with respect to all the Atlantic samples (1-7, 10-18) including those immediately adjacent to NE Atlantic waters off the Iberian Peninsula and the NW African coast. In addition, evidence of differentiation of the North Atlantic samples (1-5) from the South Atlantic samples (13-18), but also including sample 12, which is located north of the 5°N

management boundary, is highlighted. Finally, linearized F_{ST} values depict a geographic gradient of differentiation from the Northwest Atlantic to the Southeast Atlantic indicative of mixing between the South Atlantic and North Atlantic populations in the central North Atlantic, Iberian, Morocco, and Western Sahara (6-7, 10-11) samples

Table 8. Matrix of migration (Nm) for North Atlantic (NA) (n=155), Mediterranean (MED) (n=142) swordfish, and South Atlantic (SA) (n=256).

	NA	MED	SA
NA	0.0000		
MED	7.2144	0.0000	
SA	8.5915	4.8516	0.0000

AMOVA. Results of hierarchical AMOVA testing (**Table 9**) of all localities identified significant population structure among groups (F_{CT}) in the analysis of three groups (F_{CT} = 0.05171, $P < 0.00001$; North Atlantic: South Atlantic: Mediterranean) with among populations within group comparisons contributing to 0.38% of the total variation as compared to 1.63% when all localities were pooled into two groups (Mediterranean and Atlantic). However, the variation among populations within groups (F_{SC}) was significant in the analysis of all localities in 3 groups ($P < 0.01$) and in 2 groups ($P < 0.001$). The significant variation among population within groups may be explained by population admixture in some of the localities. Exclusion of localities in areas of potential mixing (6, 10-11) further reduced the variation (0.18%) among populations

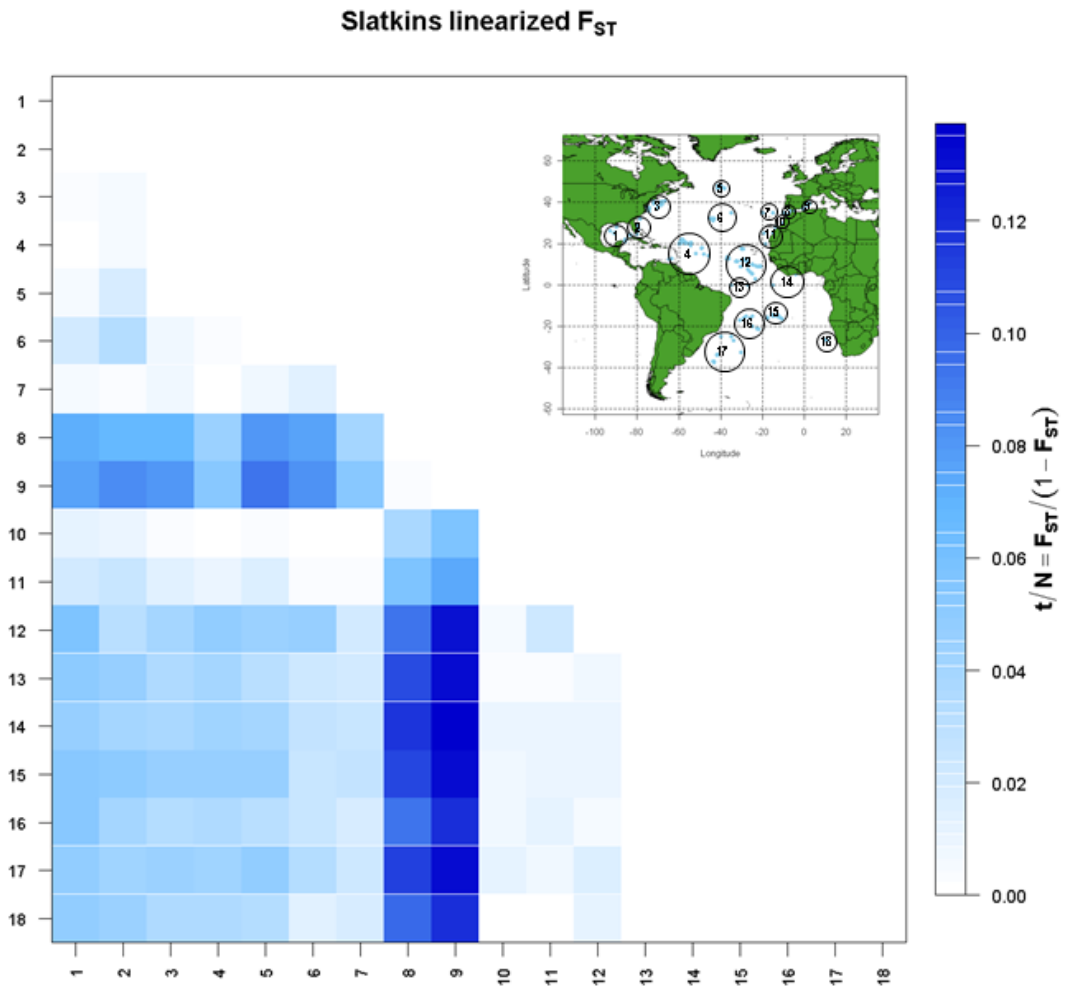

Figure 7. Slatkin's (1993) linearized F_{ST} values of 18 localities of Atlantic and Mediterranean swordfish. Population numbers correspond to the sampled localities in Table 5

Table 9. Hierarchical analysis of molecular variance (AMOVA) of 18 populations of swordfish.

	All localilities			Without localities in mixing areas		
Genetic Structure	**Variance component**	**% of total**	**Fixation index**	**Variance component**	**% of total**	**Fixation index**
	Two Groups					
	G1: Atlantic (1-7,10-18) G2: Mediterranean (8-9)			G1: Atlantic (1-5, 10-18) G2: Mediterranean (8-9)		
Among groups	0.14991	6.63	F_{CT}=0.06634**	0.16037	7.05	F_{CT}=0.07046**
Among populations within groups	0.03691	1.63	F_{SC}=0.01749***	0.04373	1.92	F_{SC}=0.02067***
Among individuals within populations	0.03735	1.65	F_{IS}=0.01802^{ns}	0.02306	1.01	F_{IS}=0.01113^{ns}
Within individuals	2.03553	90.08	F_{IT}=0.09920***	2.04876	90.02	F_{IT}=0.09981***
	Three Groups					
	G1: North Atlantic (1-5,10-11) G2: Mediterranean (8-9) G3: South Atlantic (12-18)			G1: North Atlantic (1-5) G2: Mediterranean (8-9) G3: South Atlantic (12-18)		
Among groups	0.11349	5.17	F_{CT}=0.05171***	0.14314	6.45	F_{CT}=0.06450***
Among populations within groups	0.00834	0.38	F_{SC}=0.00401**	0.00430	0.19	F_{SC}=0.00207^{ns}
Among individuals within populations	0.03735	1.70	F_{IS}=0.05171^{ns}	0.02306	1.04	F_{IS}=0.1113^{ns}
Within individuals	2.03553	92.75	F_{IT}=0.07253***	2.04876	92.32	F_{IT}=0.07683***

ns = P > 0.05, * = P < 0.05, ** = P < 0.01, *** = P < 0.001

within groups in the analysis of 3 groups and was non-significant, whereas for 2 groups the variation increased (1.91%) while remaining highly significant ($P < 0.001$) (Table 9). Removal of these potential mixing areas also reduced the significance of the two group F_{CT} ($P < 0.05$) index, while the three group F_{CT} remained highly significant ($P < 0.001$). Of the different AMOVA groupings tested variance among populations within groups was the lowest when the Iberian (6) samples were grouped with the North Atlantic and the Morocco and Western Sahara (10-11) samples grouped with the South Atlantic.

Principal coordinate analysis. The PCoA of the standardized covariance of pairwise population genetic distances provided concordant evidence of the locality assignment to at least three separate stocks (**Figure 8**). The first three axes accounted for ~94.2% of the variation among populations with Eigen values of 0.797 and 0.317 for the first and second axes respectively. Three general groups were easily identified by the analysis corresponding to the North Atlantic, South Atlantic, and Mediterranean populations, with loadings in the first axis contrasting the Mediterranean from the South Atlantic, whereas the second axis contrast the Mediterranean with the North Atlantic. The North Atlantic, while not as tightly grouped as other populations, is comprised of the Gulf of Mexico (1), Southeast U.S. Coast (2), Northeast U.S. Coast (3), Lesser Antilles (4), and east of Flemish Cap (5). The Mediterranean group consisted of the sample collected west of the Strait of Gibraltar (8) and the Mediterranean sample (9) from the Alboran Sea. Finally, the South Atlantic group included Cape Verde (12), a sample collected north of the current boundary between northern and southern stocks of Atlantic swordfish that

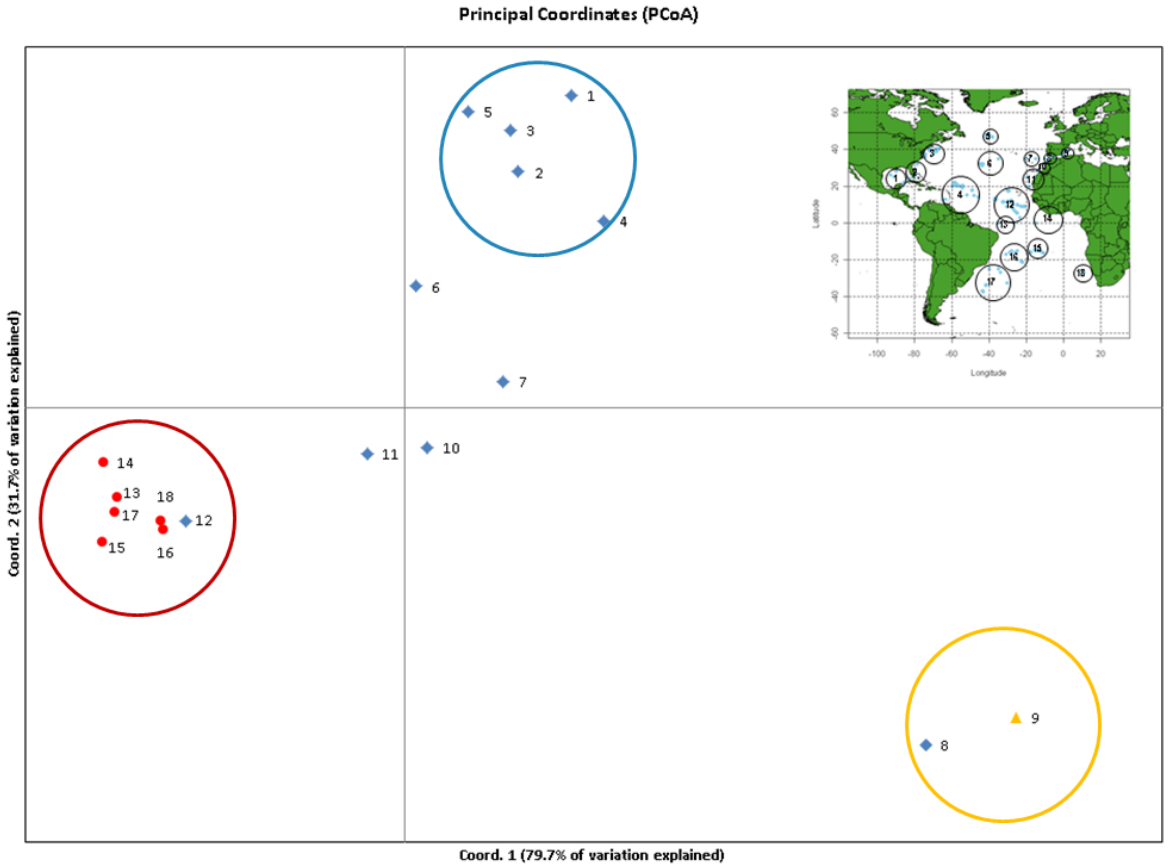

Figure 8. Principle coordinate analysis (PCoA) (Orlóci 1978) of 18 localities of Atlantic and Mediterranean swordfish. The numbered population means, corresponding to the sampling localities in the inset map, are identified by their *current management stock* (yellow triangle = Mediterranean, blue diamond = North Atlantic, red circle = South Atlantic) as defined by the ICCAT management boundaries at 5°N and the Strait of Gibraltar. Colored circles of equal diameter are drawn to display population groupings, red = South Atlantic, blue = North Atlantic, yellow = Mediterranean. In the inset sampling map black circles correspond to sampling locations and dots may include multiple individuals.

clusters tightly with Equatorial Brazil (13), Gulf of Guinea (14), Central South Atlantic (15), Brazil (16), Brazil-Uruguay (17), and Namibia (18). The remaining samples, namely central North Atlantic (6), Iberian (7), Morocco (10), and Western Sahara (11) occupy an intermediate position, with central North Atlantic (6) and Iberian (7) samples associating more closely with the North Atlantic group, whereas Morocco (10) and Western Sahara (11) more closely with the South Atlantic group.

Bayesian genetic clustering analyses

STRUCTURE analysis. Evaluation of the mean posterior probabilities from multiple STRUCTURE analyses (K = 1-10) revealed that the mean ln P(D) increased from K = 1-3 and then sharply decreased when $K \geq 4$ (**Figure 9A**) indicating that K = 3 reflects the major genetic structure in our dataset (Pritchard *et al.* 2000). Whereas when ΔK's for the data set were calculated, the peak ΔK was detected at K = 2 (ΔK_2 = 35.887) though K = 3 was very similar (ΔK_3 = 33.6548) (**Figure 9B**). To test whether the strong genetic differentiation between samples from the Mediterranean Sea and the Atlantic Ocean was leading to the underestimation of K, localities clustering in the Mediterranean (8-9) were removed from a subsequent analysis. Evaluation of only North and South Atlantic population structure identified the ΔK peak at K = 2 (not shown) indicative of genetic heterogeneity between the North and South Atlantic populations. Therefore, estimation of K using the *ad hoc* evaluation of mean posterior probabilities from multiple analyses of K (Pritchard *et al.* 2000*)* proved more effective than the ΔK approach of Evanno *et al. (2005).*

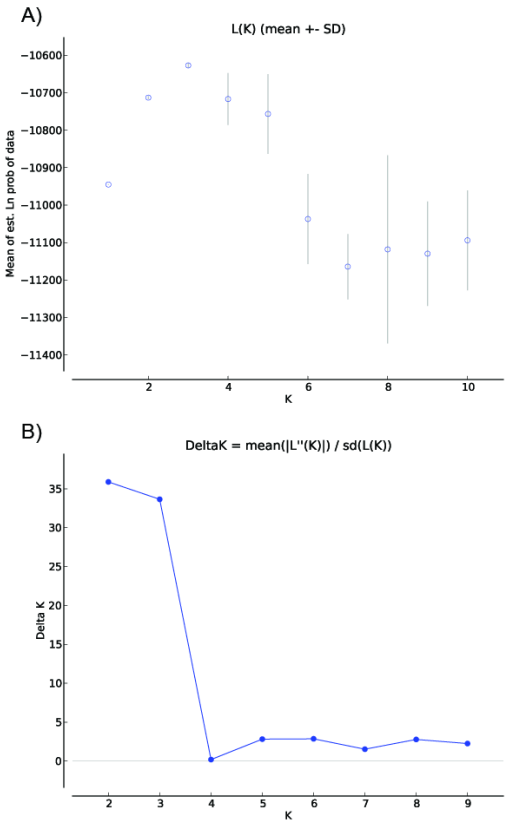

Figure 9. Estimation of the number of clusters (K) in STRUCTURE v2.3 (Pritchard et al. 2000) analysis of Atlantic and Mediterranean swordfish using (A) the ad hoc approach of Prichard et al. (2000) and the mean posterior probability of the data (L(K)) and (B) ΔK approach of Evanno et al. (2005). Twenty independent runs for each K value (1-10) were performed using 100,000 MCMC iterations with a burn-in period of 100,000.

Individual assignment of individuals (**see A-14**) at $K = 3$ was concordant with results from PCoA, AMOVA, and F_{ST} analyses (**Figure 10**). Localities 1-5 had average values of ancestry probabilities ($\bar{Q}$) belonging to the North Atlantic > 0.90 with no individuals of South Atlantic or Mediterranean origin. Swordfish collected in the central North Atlantic (6) clustered between the North Atlantic and the South Atlantic indicating

a potential mixing area, however sample size was small (n=20). The Iberian (7) sample clustered slightly closer with the North Atlantic ($\overline{Q}$ = 0.55) and represented an area of mixing with at least on individual belonging to the Mediterranean with the rest to either the North Atlantic or the South Atlantic. The sample from the area west of the Strait of Gibraltar (8) clustered ($\overline{Q}$ = 0.85) with the Mediterranean (9), which in turn contained no individuals of either North Atlantic or South Atlantic origin. The area off the Atlantic coast of Morocco (10) was also identified as an area of mixing with individuals of North Atlantic and South Atlantic ($\overline{Q}$ = 0.52) origin. Although the sample of Western Sahara (11) contained a majority of individuals assigned to the South Atlantic ($\overline{Q}$ = 0.57) it is a mixing zone with individuals of North Atlantic origin. Individuals from Cape Verde (12) together with all the individuals from the samples south of 5°N (13-18) were assigned to the South Atlantic ($\overline{Q}$ > 0.95) with no individuals from the North Atlantic or the Mediterranean. Analysis using only CaM, found that while this locus is highly informative for population differentiation between South and North Atlantic populations, assignments using only CaM failed to differentiate between the North Atlantic and Mediterranean populations. Additionally, analysis of all loci excluding CaM was able to distinguish among populations, although posterior probabilities were greater between North Atlantic and South Atlantic swordfish when CaM was included.

GENELAND analysis. The *K* value with the greatest density on the MCMC chain after a burn-in of 10,000 was *K* = 4. However, inspection of map of posterior probabilities identified that one cluster as a 'ghost' with no associated samples (not shown). Further,

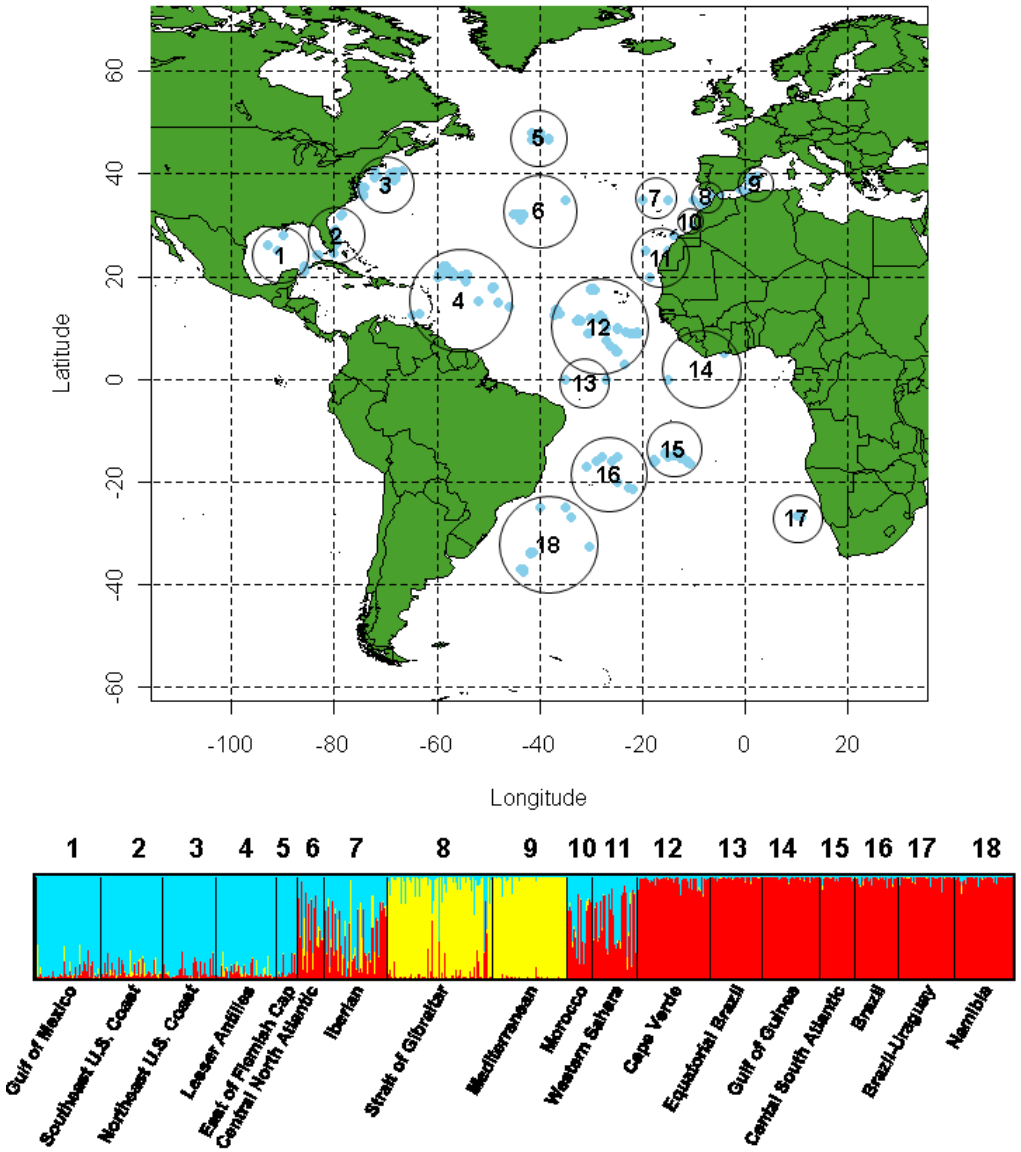

Figure 10. Bayesian individual assignment of Atlantic and Mediterranean swordfish in STRUCTURE v2.3 (Pritchard *et al.* 2000) using no admixture, correlated alleles, and LOCPRIOR models and inferred from 50 independent runs of $K = 3$ using 100,000 MCMC iterations and a burn-in period of 100,000. Estimated individual membership coefficients ($\hat{Q}$) are sorted by sampling locality and correspond to the numbered localities in the sampling map. Black circles in sampling map correspond to sampling locations and may include multiple individuals.

this ghost cluster was unstable, as it's placing or presence differed among replicate runs. The existence of 'ghost' clusters has been reported as an artifact of GENELAND which tends to overestimate *K* in a comparison to other Bayesian genetic clustering algorithms (Chen *et al.* 2007). Accordingly, GENELAND was run with $K = 3$, as identified by STRUCTURE. Localities 1-5 and part of 7 comprised the North Atlantic ($\overline{Q} > 0.90$), localities 8 and 9 the Mediterranean ($\overline{Q} > 90$), and localities 12-18 the South Atlantic ($\overline{Q} > 0.90$). The individual posterior probabilities assigned in GENELAND (**Figure 11**) identified the region corresponding to the central North Atlantic (6) and Western Sahara (11) samples as areas of mixing between the North and South Atlantic populations. The region corresponding to the western Mediterranean including the area west of the Strait of Gibraltar (8), were identified as Mediterranean, whereas the region corresponding to the Atlantic sample of Morocco (10) was identified as an area of mixing with individuals of Mediterranean and North Atlantic origin. The Iberian sample (7) is comprised of three subsamples, at 35°N 20°W (n=19), 35°N 15°W (n=5), and 35°N 10°W (n=27). The spatial and genetic models in GENELAND calculated posterior probabilities > 90% in individuals from 35°N 20°W and 35°N 15°W to the North Atlantic. However posterior probabilities calculated for individuals at 35°N 10°W were mixed between the North Atlantic and Mediterranean.

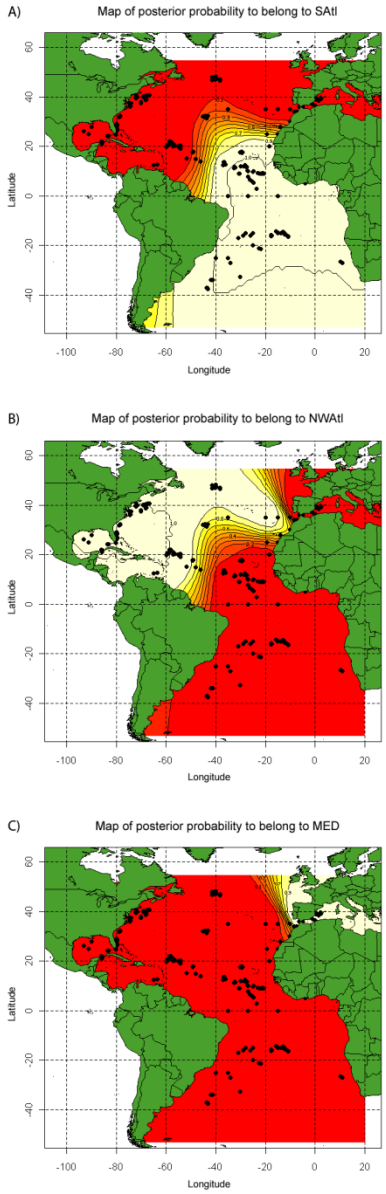

Figure 11. Posterior probability contour maps of Atlantic and Mediterranean swordfish calculated in GENELAND (Guillot *et al.* 2005) with an uncertainty on coordinate value = 30°, correlated allele frequency and spatial models, and inferred from 20 independent runs of $K = 3$ using 100,000 MCMC iterations and a thinning of 100. Posterior probability contours range from 1.0 in light yellow to 0.1 in red for membership to the (A) South Atlantic population vs. all other populations, (B) North Atlantic vs. all other populations, and (C) Mediterranean vs. all other populations. Black circles correspond to sampling locations and may indicate more than one individual. Posterior probability contours in areas of limited and/or no sample coverage are extrapolations and may therefore contain error (e.g. no northern Portugal sampling extends the Mediterranean posterior probability of membership).

Discussion

Nuclear loci evaluation

Examinations of genetic population structure have traditionally relied on neutral markers as adaptive markers may bias estimates of migration rates and effective population sizes (see Avise 2004). This study sought to identify candidate markers in non-coding nuclear introns to minimize the potential of selecting adaptive loci for multi-locus analysis which may bias our results. Of the ten nuclear markers used, nine are SNPs contained in introns and one (ATPsβ) is a third codon position synonymous SNP in an exon. While introns are often considered non-coding and neutral, introns have been reported to contain transcription regulation or splicing control elements that alter gene expression (Nott *et al.* 2003). Introns may also experience purifying and/or positive selection (Gazave *et al.* 2007) or the effects of hitch-hiking (i.e. linkage between a non-coding variant and a gene under selection) as reported in Atlantic cod (Nielsen *et al.* 2006). Therefore deviations from HWE, linkage disequilibrium, and outlier analyis are utilized to infer whether loci are potentially adaptive. In our study exact HWE (Guo & Thompson 1992) and linkage equilibrium (Raymond & Rousset 1995a) tests were not significant after Bonferroni corrections. Initially, the *CaM* locus was identified as a potential locus under selection in the outlier analysis (Beaumont & Nichols 1996) when all localities were evaluated. Restricting the outlier analysis to samples from breeding areas of Mediterranean Sea, NW Atlantic and South Atlantic Ocean yielded no outlier loci, including *CaM*.. Alvarado Bremer et al. (2005a) in comparing swordfish mtDNA CR-I lineages in the Atlantic Ocean reported the greatest levels of differentiation (i.e.,

largest Φ_{st} values) between samples from spawning areas. When the analysis was restricted to samples from spawning areas, the F_{ST} values for all loci increased as well as the H_e for several loci (e.g. Act2α, CaM, Mlc2). This result indicates that samples from feeding grounds in the NW Atlantic and the South Atlantic Ocean (i.e., Gulf of Guinea) may contain population admixture. It should be noted that in the current study the number of loci is small (n=10) which may increase the associated error in the calculation of global neutral F_{ST}. The characterization of a larger number of loci, and larger sample sizes from spawning areas are needed to determine whether *CaM* is a locus under selection. Future studies utilizing genomic technologies would yield greater numbers of loci to conduct more conclusive outlier tests. Furthermore, an alternative position has been to utilize outlier loci to provide insight into fine-scale population connectivity, admixture, mixed stock analysis, and local adaptation useful for conservation or resource management (see Allendorf *et al.* 2010; Nielsen *et al.* 2009). Studies utilizing adaptive loci in Atlantic cod (Skarstein *et al.* 2007), Atlantic herring (Andre *et al.* 2011), and Atlantic salmon (Freamo *et al.* 2011) reported that fewer loci and smaller sample sizes were required for detecting population differentiation in marine fishes when using outlier loci as compared to neutral loci. While the results of the loci ranking analysis identified CaM as the most informative locus for distinguishing between North and South Atlantic swordfish populations, the results of STRUCTURE individual assignment without the CaM locus (see Appendix 19) was still able to discriminate between North and South Atlantic swordfish. Therefore, while Cam is identified a highly informative marker for population discrimination between North Atlantic and South Altantic swordfish, this

locus alone is not capable of explaining the high resolution of population differentiation reported in this study among North Atlantic, South Atlantic and Mediterranean swordfish. In fact, the inclusion of the additional loci increases the power of population discrimination.

Atlantic swordfish population differentiation

The genetic stock structure of Atlantic swordfish, as inferred in this study with multilocus nuclear markers, includes the existence of at least three swordfish populations: the Mediterranean, Northwest Atlantic, and South Atlantic, that are independent with a small number of migrants (Nm) estimated among populations (4.85 – 8.59 per generation) indicative of limited gene flow (**Table 8**). The independence of these three populations is supported by linearized pairwise F_{ST} analyses (Slatkin 1993; Weir & Cockerham 1984), AMOVA (Excoffier *et al.* 1992), PCoA (Orlóci 1978), and Bayesian genetic clustering using both in STRUCTURE (Falush *et al.* 2003; Hubisz *et al.* 2009; Pritchard *et al.* 2000) and GENELAND (Guillot *et al.* 2005). Though no geographic barriers prohibit gene flow among Atlantic and Mediterranean swordfish populations, this study found that the spawning grounds and feeding grounds of the three populations is segregated with no evidence of migrants in any of these locales. The North Atlantic population is discrete and extends from the spawning areas in northwestern Atlantic tropical waters (Govoni *et al.* 2000; Palko *et al.* 1981) to 50°W. In the feeding areas north of 40°N the range extends to the Azores and may, according to the GENELAND, extend as far as 15°W. The South Atlantic population extends from

50°S through the South Atlantic tropical and equatorial spawning areas (Alvarado Bremer *et al.* 2005a) to 20°N at 40°W and 25°N along the African coast.

The Mediterranean population similarly may extend as far as 8°W with little evidence of Atlantic swordfish admixture within the Mediterranean Sea. These results are concordant with the phylogeographical association of mtDNA lineages previously reported (Alvarado Bremer *et al.* 2005a; Alvarado Bremer *et al.* 1996; Alvarado Bremer *et al.* 1998; Chow *et al.* 1997). The Mediterranean was identified here as the most divergent of the three populations, and these findings are in accord with other studies using mtDNA and nuclear markers (see Alvarado Bremer *et al.* 2007 for summary) as well as microsatellites (Kasapidis *et al.* 2007; Kotoulas *et al.* 2007) and provides support for the Mediterranean swordfish historical demography conclusions of Alvarado Bremer *et al.* (2005b) in which Pliestocene glacial/inter-glacial events resulted in population isolations and expansions.

The large divergence of the Mediterranean population resulted in an underestimation of the number of clusters ($K = 2$) when using the ΔK approach of Evanno et al. (2005). While Evanno's ΔK method seeks to detect the uppermost hierarchical level of population structure the method is less reliable at lower levels of genetic differentiation (Waples & Gaggiotti 2006) and may therefore underestimate *K*. When only the localities from the North and South Atlantic were evaluated the peak ΔK was estimated at two clusters indicating that there was substantial differentiation between these populations. Therefore, when analyzing datasets where the divergence of one or more populations is disproportionately higher than the divergence among the

remaining populations, a careful evaluation of ΔK is required so as to not underestimate K. In such instances the posterior probability estimate is preferred.

The results of Bayesian clustering analysis are not coinsistent with recent analyses of global swordfish population structure using microsatellites (Kasapidis *et al.* 2008; Kotoulas *et al.* 2007) in which levels of differentiation between North and South Atlantic were found to be extremely low thus precluding the use of individual clustering assignment. Addressing the low differentiation ($F_{ST} \approx 0.0015$) Kasapidis *et al.* (2007) argued that estimates of differentiation identified with nuclear SNPs (e.g. *CaM*) were due to adaptive selection. This explanation, however, fails to explain the concordance between estimates of differentiation derived from mtDNA and nuclear SNPs. Yet in individual assignment based on Bayesian statistics in this study all of the larvae (n=52) from the Gulf of Mexico were concordant with the genetic signature of adult swordfish from North Atlantic spawning and feeding grounds. This further validates that the signal of differentiation between North Atlantic and South Atlantic is the consequence of population subdivision and levels of gene flow at levels sufficiently low to prevent panmixia in this basin. The low level of differentiation within the Atlantic reported by Kasipidis et al (2007) using microsatellites compared to this study using nuclear markers may be associated with populations with large effective population sizes combined with high mutation rates resulting in microsatellite allele size homoplasy and thus in a diminishing signal of differentiation. Microsatellite allelic richness and heterozygosities in marine fishes appear to be substantially higher compared to anadromous or freshwater fishes and may be explained by higher mutation rates in marine fishes (DeWoody &

Avise 2000). This inverse relationship between microsatellite polymorphism and F_{ST} was reported in walleye pollock (*Theragra chalcogramma*) (O'Reilly *et al.* 2004) which have similar population demographics to swordfish (e.g. large effective population sizes, population structure, high migratory potential). Therefore, in order to detect differentiation using microsatellite loci, either very large sample sizes or a large number of loci would be required. It should also be noted that the microsatellite analyses of Atlantic swordfish (Kasapidis *et al.* 2007; Kasapidis *et al.* 2008; Kotoulas *et al.* 2007) relied on a small number of microsatellies (4-10) and that many of the samples used for analysis of the North Altantic are associated with areas that our analysis identifies as areas of mixing.

Conflicting estimates of population differentiation estimates between microsatellite and mtDNA markers has been reported in other highly migratory fishes and has often interpreted as evidence of male mediated gene flow (e.g. Buonaccorsi *et al.* 2001). However, in swordfish and certain istiophorids (i.e., blue marlin) females are the larger and occur in higher numbers at higher latitudes where males are rarely recorded (Palko *et al.* 1981). It would therfore be highly unlikely that gene flow directed by males would account for inter-oceanic allele frequency discrepancies as these temperate migration corridors are mostly inhabited by large females. In addition, the levels of differentiation between North and South Atlantic populations reported with mtDNA (F_{ST} = 0.0244)(Alvarado Bremer *et al.* 2005a) are lower than those reported in this study (F_{ST} = 0.0393), thus providing no evidence of male mediated gene flow.

Atlantic swordfish population subdivision and admixture

While previous studies addressing Atlantic swordfish population admixture had identified areas of potential mixing between the North Atlantic and Mediterranean populations (Alvarado Bremer *et al.* 1998; Viñas *et al.* 2007) and between the North Atlantic and South Atlantic populations (Alvarado Bremer *et al.* 2005a; Chow *et al.* 2007; Chow & Takeyama 2000) no estimates of population admixture have been provided. The current management boundary utilized by ICCAT between the Mediterranean Sea and the North Atlantic Ocean is the Strait of Gibraltar. Of the 83 individuals sampled immediately to the west of the Strait of Gibraltar (8) 93% were assigned to the Mediterranean in the STRUCTURE analysis. Of the remaining individuals, three swordfish (3.6%) were identified as North Atlantic and another three (3.6%) as South Atlantic. Four Mediterranean swordfish were also identified in the Iberian sample (7) as follows: three at 35°N 10°W and one at 35°N 20°W, representing approximately 11% and 5% of the total number of individuals sampled from the two locations respectively. The GENELAND consensus results (**Figure 11C**) identified the Mediterranean stock extending past the management boundary into the North Atlantic to around 8°W. It should be noted that the mapping algorithm of the GENELAND results extrapolates the posterior probability maps into areas of limited sample coverage. Since we had no samples off the northern coasts of Portugal or Spain the posterior probability curves in this area should not be interpreted to mean that the Mediterranean stock extends around the European continent in the Bay of Biscay and the Celtic Sea (**Figure 11C**) as microsatellite analysis of samples off the northern coast of Portugal identifies

mostly Atlantic swordfish (Kotoulas *et al.* 2007). This would indicate that Mediterranean swordfish primarily inhabit the shallower Northwest African and Iberian shelf waters in the Atlantic proximal to the Strait of Gibraltar. Occasionally migrants may extend farther into the Northern Atlantic towards the Azores but are not expected to be in high abundance. Movements of mature swordfish in the North Atlantic into the Mediterranean through the Strait of Gibraltar occurs from May to the beginning of July, putatively associated with spawning behavior, have been documented (de la Serna & Alot 1990). Later in the year, between August-November a migration associated with feeding behavior in the opposite direction (east to west) across the Strait takes place with no reproductively active swordfish and with 15% of the catch corresponding to juveniles. As our samples were acquired during these reported summer and fall oscillations, we are unable to confirm a contraction of Mediterranean swordfish in these areas during the winter and spring. No Atlantic swordfish were identified within the Mediterranean Sea during a larger sampling period that included some winter sampling. Bayesian analysis of microsatellite data from 602 Mediterranean swordfish identified only three individuals of Atlantic origin (Kotoulas *et al.* 2007). Similarly, using mtDNA CR-I sequence data Alvarado Bremer et al. (1999) documented no Atlantic swordfish east of the Strait of Gibraltar. Bayesian analyses of the Mediterranean sample with STRUCTURE and GENELAND in this study support the limited presence of Atlantic swordfish in the Mediterranean Sea. This would suggest that oceanographic and or behavioral cues lead to an avoidance of the Mediterranean Sea by Atlantic swordfish and limit the majority of the Mediterranean admixture to the shallower waters of both the

northwest African and the Iberian Atlantic shelves. It should be noted that in the GENELAND analysis the 30° uncertainty value was correlated with known migration behaviors of Northwestern Atlantic swordfish and that Viñas *et al.* (2010) reported evidence of genetic differentiation between eastern and western Mediterranean swordfish suggesting that the 30° uncertainty value may be too large for the Mediterranean samples.

While the admixture between the North Atlantic and Mediterranean swordfish could be constrained by the geographical barrier of the Strait of Gibraltar, no such physical barrier exists between the North Atlantic and South Atlantic, and consequently admixture occurs over a considerably broader geographic area. The current management boundary separating the North Atlantic from the South Atlantic populations at 5°N was challenged by Chow *et al.* (2007) on the basis of significant differences in allele frequency of the nuclear locus *CaM*, who suggested that South Atlantic swordfish extended to about 15°N. The results of our analyses support the hypothesis of a northern extension of the South Atlantic swordfish stock; however a horizontal 15°N boundary is too simplistic to fully address the extent of admixture both regarding the latitudinal position and the shape of the boundary. Examination of the results of PCoA (**Figure 9**), STRUCTURE (**Figure 10**), and GENELAND (**Figure 11**) provide excellent visualizations of the subdivision between northern and southern populations and of the extent of admixture. These results strongly indicated that the South Atlantic swordfish stock extends farther north, past Cape Verde (12) at around 20°N. However, instead of a horizontal boundary across the basin extending from the Caribbean Sea to the African

coast, the results of GENELAND depict a boundary more akin to a step that extends north from about 0°N 45°W to 25N 45W, and from there as a nearly horizontal boundary east towards the African coast (**Figure 11**). To the north and west of this boundary, an admixture zone begins around Western Sahara (11) and continues north to Morocco (10) and the Iberian sea (7) extending west towards the central North Atlantic (6) and then south towards the northern coast of Brazil. However, the extent of admixture between northern and southern stocks is not symmetrical. While the presence of South Atlantic swordfish is well documented past the boundary zone, no swordfish of North Atlantic origin were identified in the South Atlantic. Furthermore, the posterior probability membership contours generated by GENELAND (**Figure 11**) could be used for mixed stock allocation in the identified areas of stock admixture.

In the absence of a geographic barrier to explain this asymmetric pattern of admixture, biological, behavioral, and oceanographic impediments to gene flow need to be examined. Conventional tag-recapture data (García-Cortés *et al.* 2003; Neilson *et al.* 2007), though biased in its coverage to primarily the Northwest Atlantic fishery, indicates that swordfish tagged west of the Azores generally follow similar patterns of directional migration, west by southwest, back to the primary breeding grounds of the North Atlantic indicative of spawning and feeding ground fidelity, whereas swordfish tagged east of the Azores (of which there are far fewer records) tend to move east by southeast. Interestingly, of the few swordfish tagged between 15°N and 30°N, movement generally leads east by northeast. It must be emphasized that while conventional tagging data may reflect some of the same molecular distribution trends

inferred in this study, these data are heavily biased to areas of high fishing effort (Neilson *et al.* 2007). Pop-up satellite archival tag (PSAT) data (Neilson *et al.* 2009; Sedberry & Loefer 2001) however has yielded similar evidence of seasonal spawning and feeding ground migrations in the Northwest Atlantic.

While spawning ground fidelity may explain the low gene flow between North and South Atlantic swordfish, it doesn't fully explain why admixture only seems to occur in the Northeast Atlantic waters east of about 50°W and south of 30°N. Review of data from the World Ocean Atlas (Garcia *et al.* 2010) in the presumed boundary area identifies a large difference in dissolved oxygen (DO) concentrations between the two oceans at depths >100 m (**Figure 12**). Nutrient upwelling, caused by prevailing winds off the western coast of Africa, creates a large hypoxic zone in the eastern tropical Atlantic (Diaz 2001). In a study of blue marlin vertical habitat use in the eastern tropical Atlantic, Prince *et al.* (2010) using PSAT data reported that dive depth maximums where significantly lower inside this oxygen minimum zone (OMZ) with individuals generally remaining above 2.5 mL L^{-1} DO. Vertical migrations by swordfish are known to be substantially deeper (Dewar *et al.* 2011) than by other billfishes, therefore swordfish distribution would be more closely associated with changes in concentrations in DO with depth. This is not to say that swordfish are impeded by crossing such a DO boundary, however as no corresponding hypoxic features in similar size or magnitude exist in the North Atlantic. Conversely, South Atlantic swordfish may be habituated to such features, behaviorally and/or biologically, and are therefore not impeded when

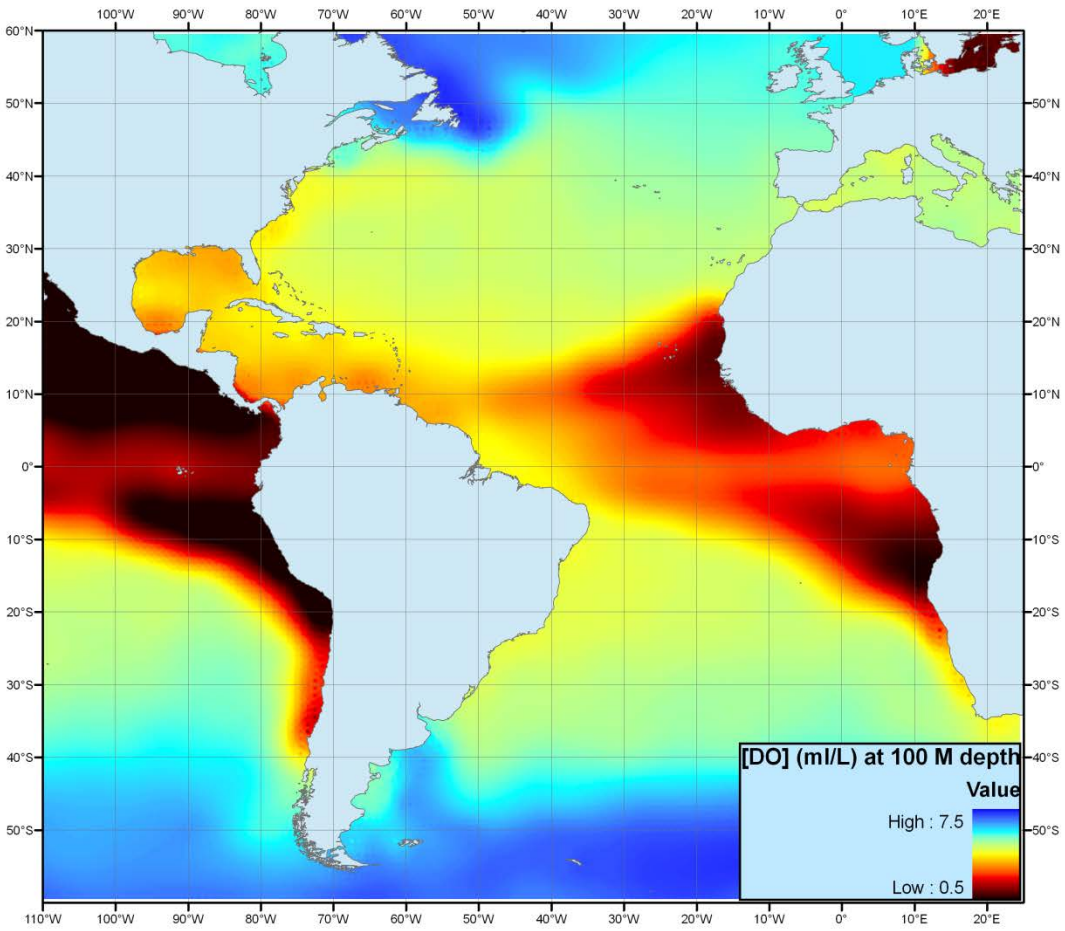

Figure 12. The oxygen minimum zone (OMZ) in the South Atlantic swordfish feeding grounds at 100 m depth. Annual mean dissolved oxygen (DO) at 100 m depths in mL L^{-1} are from the World Ocean Atlas 2009 (Garcia *et al.* 2010). The OMZ depicts a similar boundary as the posterior probability of membership contour maps in Figure 11.

transitioning through such a boundary thus accounting for the significant admixture in the Northeast Atlantic. A cursory comparison of **Figures 11** and **12**, particularly on African coast where the beginning of North and South Atlantic swordfish admixture corresponds geographically with the OMZ, identifies the need to further investigate a possible relationship between [DO] and genetic population structure. Other oceanographic features are known to acts as barriers to dispersal in proximity to coastal areas. For instance while recognizing that the Amazon discharge would not be expected to affect the movements of adult highly migratory pelagic fish, Chow *et al.* (Chow *et al.* 2007) hypothesized that the discharge may act as a freshwater barrier to passive larval dispersal between the Caribbean and Brazilian provinces. Interestingly, the resulting GENELAND posterior probability of membership contours coincide geographically with this feature as well as were an inferred break in potential spawning (Alvarado Bremer *et al.* 2005a) has been reported (**Figure 13**). Further, the same region coincides with areas where historical catches of adult swordfish are low. If adult catches properly reflect abundance in this region (Figure 8), then the low density of adults together with the Amazon discharge preventing the admixture of larvae from northern and southern breeding areas, as hypothesized by Chow et al. (2007), could together promote the observed patterns of genetic differentiation between northern and southern populations of swordfish. Finally, the Atlantic swordfish population structure may be influence by sea surface currents as other pelagic species have been reported to closely associate with such features (e.g. blue marlin, Seki *et al.* 2002). The North Atlantic gyre, the canary current, and the Atlantic equatorial counter current all associate with population

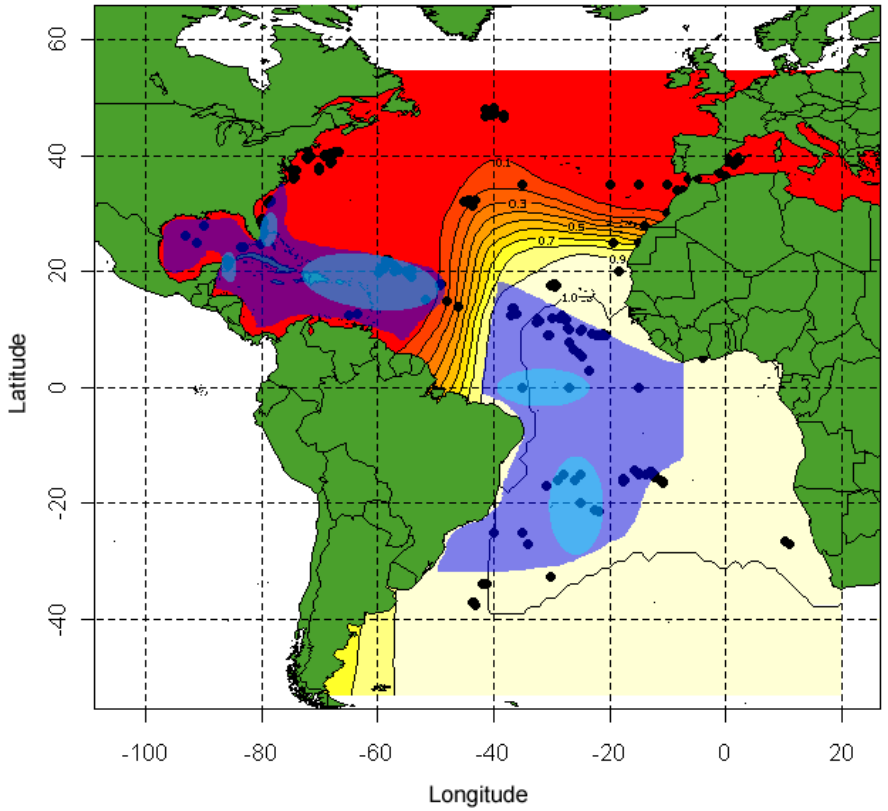

Figure 13. GENELAND map of posterior probability of membership to the South Atlantic population overlaid the regions of reproductive of Atlantic swordfish summarized by Alvarado Bremer *et al.* (2005a). Shaded in dark blue are the Northwest Atlantic and South Atlantic areas where eggs and or early life history stages have been collected. The areas shaded in lighter blue correspond to areas where females with high-gonadal indices and/or hydrated oocytes have been collected that coincide to areas of biased male to female sex-ratios (Mejuto *et al.* 1998).

structure of North and South Atlantic swordfish and may also explain why population admixture appears to occur primarily in Northeast Atlantic waters.

Implications for fishery management and future research

Based on the findings reported in this study, the current ICCAT management boundaries at 5°N and at the Strait of Gibraltar for Atlantic swordfish need to be revised. While Chow *et al.* (2007) proposed moving the boundary between the North and South Atlantic stocks to around 10°N-20°N, the results of this study suggests that such placement of the boundary may be too conservative. Based on GENELAND results, a revised boundary proposed in this study between North Atlantic and South Atlantic swordfish (**Figure 14**) that extends as far north as 25°N but only comprising the waters to the east of roughly 45°W. The boundary between the Mediterranean Sea and the Atlantic Ocean should be revised to include the Mediterranean swordfish that were identified west of the Strait of Gibraltar and east of 10° W. Since few Mediterranean swordfish were identified west of 10° W, and since the precise coordinates of capture in these areas were limited to 2.5° x 2.5° quadrant, revision of the boundary to 8° W so as to include only the shallower waters of both the northwest African and the Iberian Atlantic shelves is recommended. Although Viñas *et al.* (2007) suggested that Mediterranean swordfish may move beyond 10°W into Atlantic water and south to 20°N along the coast of Morocco, detailed sampling an characterization with mtDNA, microsatellites, and SNPs may resolve this issue. There is also the need for mixed stock allocation in the Northeast Atlantic as these areas are identified as heavily mixed.

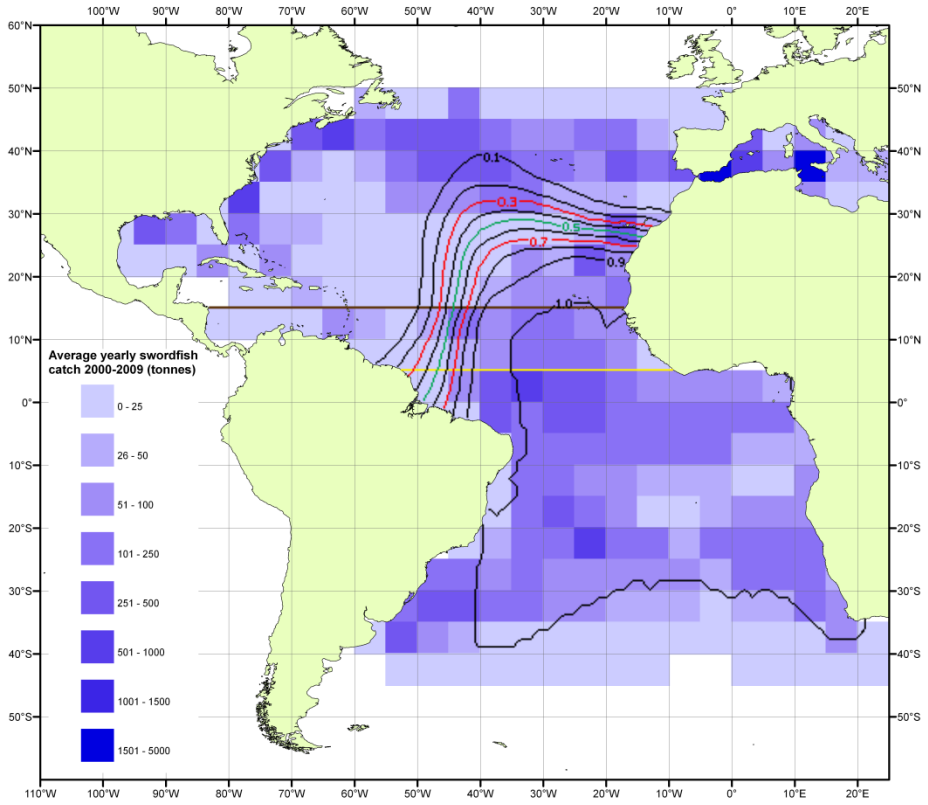

Figure 14. Map of average yearly longline commercial catches of Atlantic and Mediterranean swordfish, in tons, from 2000 – 2009 (FAO, 2011). The current management boundary at 5°N (yellow line), the proposed boundary of Chow *et al.* (2007) (brown), and the GENELAND posterior probability contours of membership to the South Atlantic are displayed. If a new management boundary is placed at 0.5 posterior probability membership contour (in green), the average yearly catches in the areas within the 0.3 and 0.7 posterior probability membership contours (in red) account for 5.7% of the total catches of North Atlantic swordfish outside of the 0.7 posterior probability membership contour

Additional sampling is needed, first, to fill in the sample coverage in areas that are not yet represented, and second, to identify any seasonal variations in the population structure. However, areas not sampled in this study correspond to areas of low fishing pressure (**Figure 14**) whereas the samples analyzed in this study are representative of the areas and seasons of highest fishing pressure. Accordingly, additional sampling efforts would be expected to only add fine scale resolution to the current findings. Average yearly Atlantic swordfish catches from 2000 - 2009 (FAO Atlas of Tuna and Billfish Catches, 2011) in the area within the 0.3 – 0.7 posterior probability of membership boundary contours estimated in GENELAND account for only 5.7% (485 tons) of the total yearly North Atlantic catches (8553 tons) concordant with the North Atlantic management boundary proposed here (**Figure 14**). Tagging studies using PSATS should be conducted in the Northeast Atlantic and in the eastern tropical Atlantic to further characterize migratory behaviors in these regions. Finally, there is a need for fisheries managers to reassess the Atlantic and Mediterranean stocks as a substantial number of South Atlantic and to a lesser extent Mediterranean swordfish, have been allocated to the North Atlantic stock.

CHAPTER V

GENERAL CONCLUSIONS AND SUMMARY

In this study, we examined: 1) the applicability of high-resolution melting analysis (HRMA) in population genetic studies of non-model organisms, 2) the development of nuclear markers in Atlantic swordfish and the methodology whereby nuclear gene variation can be quickly screened, identified, and genotyped using short-amplicon (SA) HRMA and unlabeled probe (UP) HRMA, and 3) the use of HRMA to characterize nuclear markers to study the genetic population structure of Atlantic swordfish and estimation of population admixture by means of Bayesian individual assignment. The general conclusions and results of these three investigations are as follows:

High resolution melting analysis in non-model organisms

In Chapter II it is demonstrated that tissue quality and/or type were not limiting factors for the use of HRMA in population genetic studies. Uniform HRMA melting curves from multiple tissue types (e.g. liver, heart, skeletal muscle, etc.) were repeatable and unambiguous, as there was no evidence of melting curves 'shifting' or confounding results. While tissue quality played an important role in the success of amplification, there was also no evidence of melting curve 'shifting' or confounding results due to differences in tissue quality.

Organic DNA extractions were not required for HRMA analysis. Since HRMA was previously used in clinical and diagnostic studies, phenol-chloroform or other organic extraction methodologies were commonly used. We used a Proteinase K digestion followed by ETOH precipitation without organics (Grieg 2000) and DNA template quality was adequate for HRMA.

The high throughput, closed-tube, low cost, and high resolution to discriminate alleles makes HRMA a desirable genotyping tool that should not be confined to just clinical and diagnostic studies but rather adopted for use in studying natural populations. Additionally, short amplicon (SA) and unlabeled probe (UP) variations of HRMA increased the resolution of allele discrimination.

Methodological workflow for development of nuclear markers using HRMA

In chapter II a methodological workflow was provided for SA-HRMA and UP-HRMA genotyping assay design and optimization. HRMA screening and Sanger sequencing of ten nuclear genes: acidic ribosomal phosphoprotein P0 (ARP), adenine nucleotide translocator (ANT), aldolase B (AldB), alpha skeletal actin 2 (Act2A), ATP synthase beta-subunit (ATPsβ), calmodulin (CaM), golgi pH regulator (GpHr), lactose dehydrogenase A (ldhA), myosin light chain (mlc2), and signal recognition particle 54 (SRP54); identified 78 SNPs and found gene introns were four times more variable than exons. The use of initial HRMA screening provided a reduction in sequencing effort for marker development.

Of the ten genes, SNPs in four (ARP, ATPsβ, CaM, and SRP54) were genotyped using SA-HRMA. Advantages of SA-HRMA over traditional HRMA for genotyping SNPs include: increased genotyping speed, reduced error associated with genotype masking, and increased ΔT_m between genotypes thereby improving genotyping accuracy. Potential disadvantages associated with SA-HRMA included: preclusion when suitable SA-HRMA primers flanking SNPs of interest cannot be identified, difficulty differentiating multiple SNPs (particularly those that are base-pair neutral), and obscured genotyping from primer-dimer formation (though this was not a problem in this analysis).

Four genes were genotyped via UP-HRMA: ANT, Mlc2, GpHr and ldhA. The number of alleles genotyped ranged from three to seven. In addition to the advantages shared with SA-HRMA, UP-HRMA is able to identify and differentiate multiple SNPs in a single closed-tube reaction, thereby potentially increasing the power to detect population differentiation without having to rely on Bayesian haplotype reconstruction methods. Furthermore, UP-HRMA can reveal new SNPs while genotyping populations that in alternative non-sequencing genotyping technologies could cause genotyping failures or errors. While the potential of cross-species utility of the swordfish specific HRMA primers was limited, as swordfish are a monotypic species, an examination of zinc finger protein sequences in sailfish (*Istiophorus platypterus*), blue marlin (*Makaira nigricans*), and Atlantic white marlin (*Kajikia albida*) identified SNPs that would preclude cross species amplification using site specific SNP technologies, yet could be genotyped using UP-HRMA.

Atlantic swordfish genetic population structure and mixed stock analysis

In chapter IV the multi-locus genetic analyses of ten nuclear loci in 774 individual swordfish from 18 localities representative of the North Atlantic, South Atlantic, and the Mediterranean, revealed the existence of at least three populations with low levels of gene flow among them. Pairwise F_{ST} values, AMOVA, PCoA, and Bayesian individual assignments using STRUCTURE and GENELAND were largely concordant with previous mtDNA and nDNA genetic studies, but provided a more detailed understanding of population structure and the levels of admixture among Atlantic swordfish populations that could not be obtained by previous studies using single loci.

The Mediterranean was identified as the most divergent of the three populations and 93% of the swordfish from the area to the west of the Strait of Gibraltar (currently managed as North Atlantic swordfish) were identified as Mediterranean swordfish. Additional Mediterranean migrants were reported in the Northeast Atlantic at low numbers as far west as 35°N and 20°W. Therefore, the current boundary of Mediterranean swordfish may need to be extended past the Strait of Gibraltar perhaps as far as 8°W to include the Mediterranean swordfish that utilize the relatively shallower waters of the Atlantic continental shelves of northwest Africa and the Iberian Peninsula as feeding grounds (de la Serna & Alot 1990).

Results indicate that the range of South Atlantic swordfish population extends to a region of North Atlantic waters west of 40°W as far north as 25°N. To the north and to

the west of that region an admixture zone between the North Atlantic and South Atlantic swordfish populations is identified where average posterior probabilities to belong to either populaton were low (50-60%). By contrast, posterior individual assignments in the Northwest Atlantic and the South Atlantic were greater than 90%.

The use of both a spatial and genetic model in GENELAND allowed posterior probability membership values to be extrapolated between samples and were used for fisheries management recommendations. The current management boundary separating the North Atlantic from the South Atlantic populations at 5°N was challenged by Chow *et al.* (2007) who suggested that South Atlantic swordfish boundary be extended to about 15°N, however a horizontal 15°N boundary is too simplistic to fully address the extent of admixture both regarding the latitudinal position and the shape of the boundary. Based on posterior probability membership contours obtained with GENELAND, this study proposes a boundary shaped as a step extending north from 0°N 45°W to 25°N 45°W and from that longitude, as a nearly horizontal line, east to the African coast. Average yearly catches of swordfish between the 0.3 – 0.7 GENELAND posterior probability membership contours immediately adjacent this newly proposed boundary account for only approximately 5.7% of the total yearly North Atlantic catch, therefore, mixed stock allocation in these areas may not be needed.

Mechanisms for genetic isolation in the absence of geographic barriers to gene flow may include: 1) spawning and feeding site fidelity which has been inferred from conventional tagging and PSAT data (García-Cortés *et al.* 2003; Neilson *et al.* 2007; Neilson *et al.* 2009; Sedberry & Loefer 2001) and by the characterization of the

reproductive biology of swordfish, including eggs and early life stages distribution, high gonadal indices, and sex-ratios at catch (summarized in Alvarado Bremer *et al.* 2005a), 2) the presence of an oxygen minimum zone (OMZ) in the eastern tropical Atlantic that corresponds geographically with the eastern edge of the proposed boundary, 3) the freshwater Amazon discharge that may act as barrier to passive larval dispersal between the Caribbean and Brazilian provinces and which also coincides geographically with the western edge of the proposed boundary, and 4) surface currents in the Atlantic Ocean.

Additional sampling, both seasonally and in areas of limited coverage, may provide additional insight to the Atlantic swordfish population structure reported in this study. Tagging studies using PSATs should be targeted in the Northeast Atlantic in areas of admixture and in the eastern tropical Atlantic in the OMZ to further identify migratory behaviors in these regions. An increase in nuclear markers as well as samples may serve to increase the resolving power of the Bayesian individual assignment tests. And finally, there is a need for fisheries managers to reassess the Atlantic and Mediterranean stocks as a substantial portion of South Atlantic and Mediterranean swordfish have been allocated as North Atlantic swordfish.

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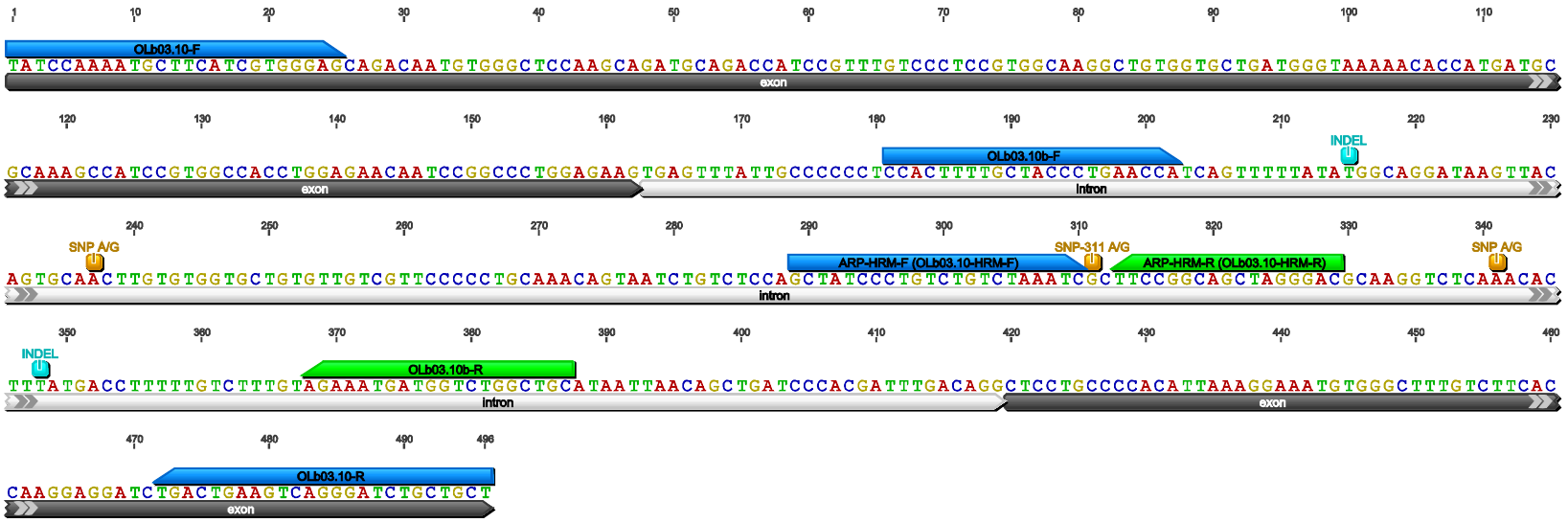
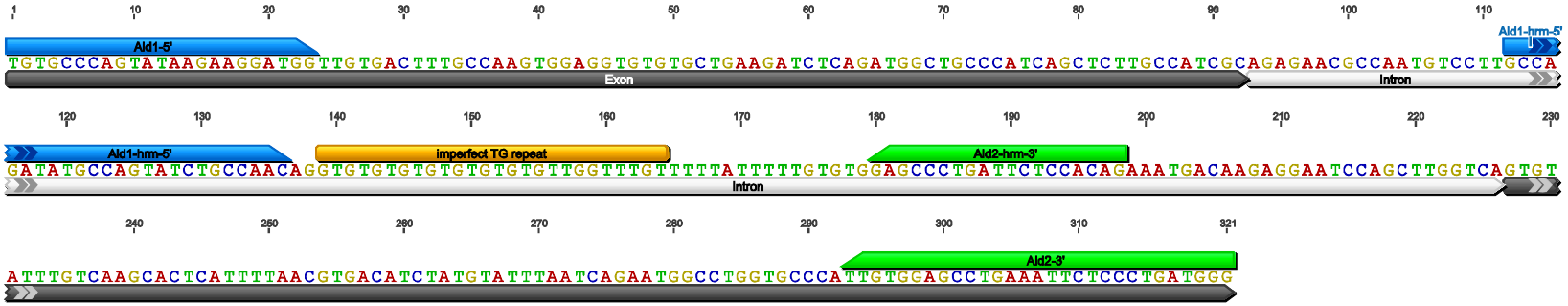
APPENDIX

A-1. Table of additional putative loci and primers that were evaluated in this study. Amplification of loci either failed or the loci were not polymorphic.

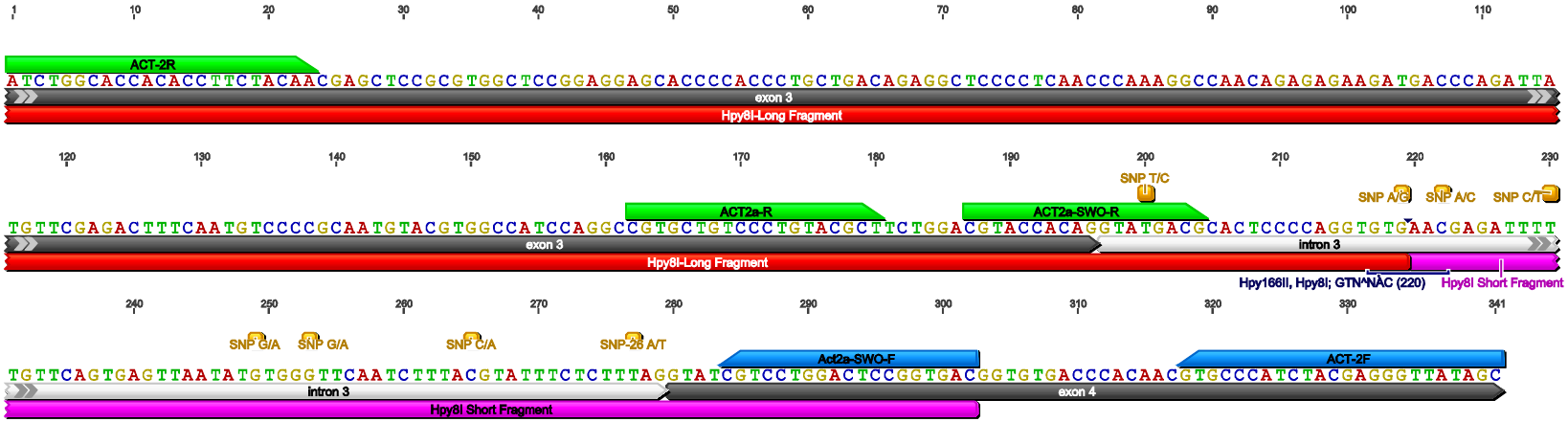
Gene	Locus	Primer Sequence 5 '-> 3'
Actin	ACT1-F	CCA TAC CTT CTA CAA TGA GCT CCG
	ACT1-R	GAC CAG AGG CGT AGA GGA GAG C
Amylase 2	Am2B-F	CCT TCA TCT TCC AGG AGG TAC
	Am2B-R	TTC ACC TCC CAG ATC AAT AAC
Chymotrypsin B	ChymB-6F	GCA TGA GGG CTG TGA CAC GGG
	ChymB-6R	ATC GTG TCC GAG GCT GAC TGC AA
Glycerol-3-phosphate	Gpd2-F	GCC ATC AAT GAC CCC TTC ATC G
	Gpd2-R	TTG ACC TCA CCC TTG AAG CGG CCG
Glucose-6-phosphate dehydrogenase	G6PD-F	GAG CAG ACG TAT TTT GTG GG
	G6PD-R	GCC AGG TAG AAG AGG CGG TT
Growth hormone	FT-71	ACC TGG TGC ATG TCC TTC TTG AAG CA
	FT-70	ACC AAC GNC TGT TCA ACA TCG CNG T
Glucocorticoid receptor	GCR-F	CTA CAG CAC CAG CAA CAT CAG
	GCR-R	GAG CTC ATG CTG CTC TGG AGA C
Isocitrate dehydrogenase	IDH2-F1	GAG ATG ACC MGG ATC ATC TGG
	IDH2-F2	ACC ATY GGC AGR CAY GCC
	IDH2-F3	TGA GCA CNT TCT TYA CCA T
	IDH2-R1	TCT TRA TKG GRT CAN GGA AGT C
	IDH2-R2	GTC CAN GCR AAR ATR CTS GCA AT
	IDH2-R3	AGT TYT TGC ARG CCC AMA CAA
Major histocompatibility complex class II	MchII-F	ACT CTA ATC TGG AGT ACA TGC
	MchII-R	CAG GAG ATC TTC TCT CCA GCC
Myosin light chain 3	Mlc-3F	AGT AAT GAC GTC GCA GAT GTT CT
	Mlc-3R	CGA CAG GTT CAC TCT CGA GGA G
Opsin	Ops-1F	GCT CAT GGG CCT GCA GAC CAC AA
	Ops-1R	CCT GCT CAA CCT GGC CAT GGC
Pyruvate Kinase	PK-1F	GAG ATC CGC ACY GGA TTR GTG AAA GGG
	PK-2F	TCT TCG CCA GCT TCA TCC GCT
	PK-3F	CAG AAG ATG ATG ATY GGA CGC TCG AA
	PK-4F	GTS GCC AAY GCT GTK CTG GA
	PK-1R	TCC AGC CGG TRA CCA CGA TCA CCA
	PK-2R	GCC TCC CTG CAG ATC GAG TGC ATC A
	PK-3R	TCC AGC CSG TYA CCA CRA TCA C
	PK-4R	TGT CSA CGT CST CRG CCC A
	PK-5R	CCR TCC AGM ACM GCR TTG GC
Rhodopsin	Rh1039-R	TGC TTG TTC ATG CAG ATG TAG A
	RH545-F	GCA AGC CCA TCA GCA ACT TCC G
Thiredoxin reductase	Tr1-F	AGG GAA CAG AGG ATG AGC TGG AC
	TR1-R	TCT CAG CTT CCT CCA GCT TGG TG
Beta Tubulin	FT-95	GAT CTT CAG ACC CGA CAA CTT T
	FT-96	ACT CTT CTC GGA TTT TGC TGA T
LTR transposon	LTRS-F1	CAA CAA CAA ACT GGC GGC GTG G
	LTRS-F2	GCC TCA AAG GAC AAG GAA AC
	LTRS-R1	TCT CTG TGA TTT CCA TCA GGT
	LTRS-R2	TCC AGG AAC TCT CCC ACC AAC TT
	LTRS-R3	CTT GAT GTT GTT GGA GTC TGT GAG GAA C
Tata box binding protein	TBP-F1	ACA CCA GCC CTC AGA GAG C
	TBP-F2	GCA GAG TAC AAC CCA AAG CTT
	TBP-R1	GAC TGC TCC TCA CTC TTG GCT CC



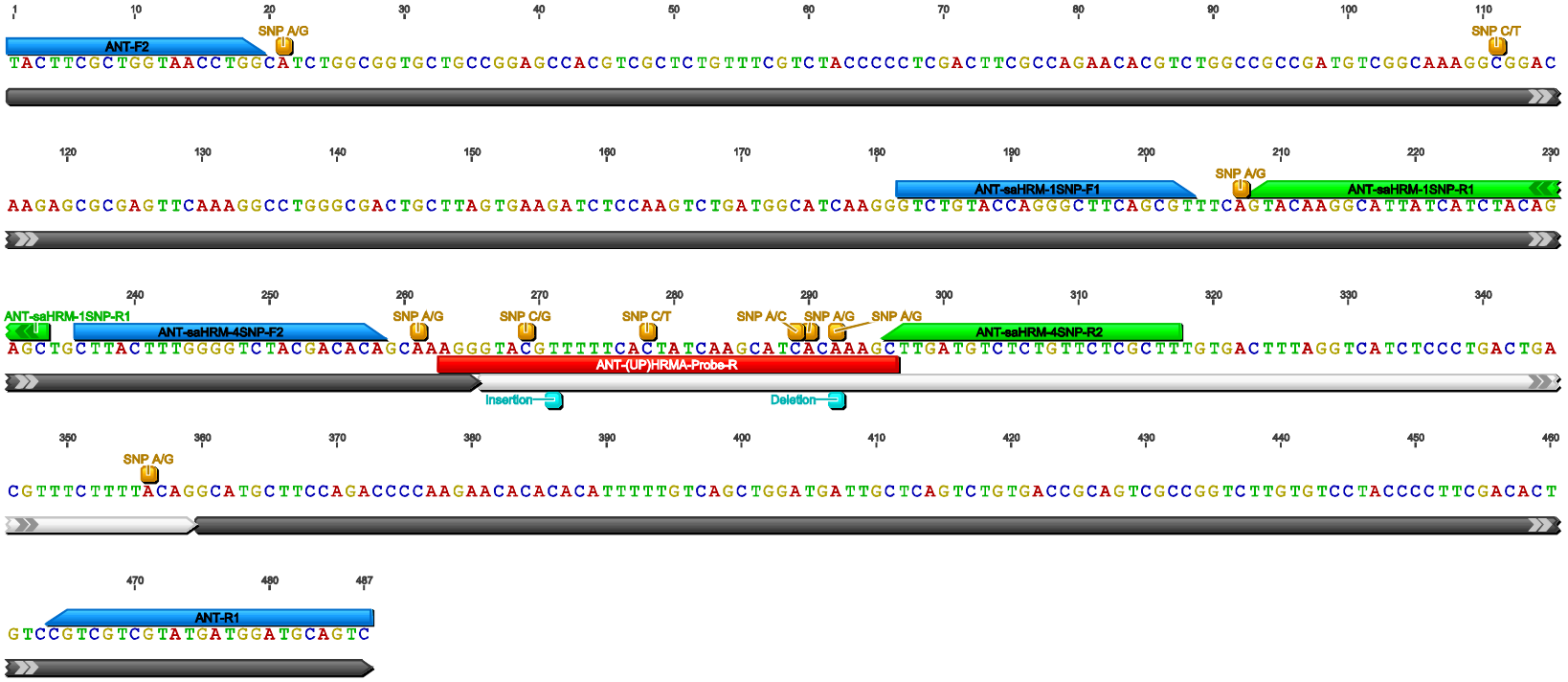
A-2. Sequence segment of the ARP locus in the acidic ribosomal phosphoprotein P0 gene with associated forward primers (blue), reverse primers (green), SNPs (orange), indels (light blue), exons (dark grey), and intron (light grey).



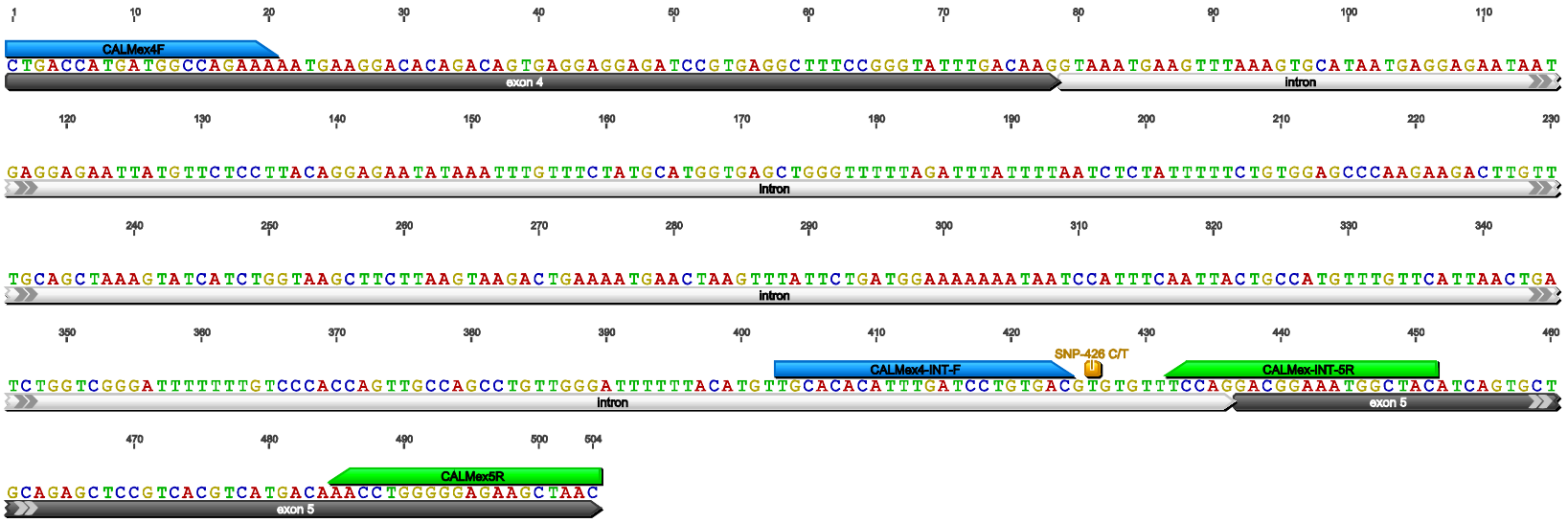
A-3. Sequence segment of the AldB locus in the aldolase B gene with associated forward primers (blue), reverse primers (green), imperfect GT repeat (orange), exons (dark grey), and intron (light grey).



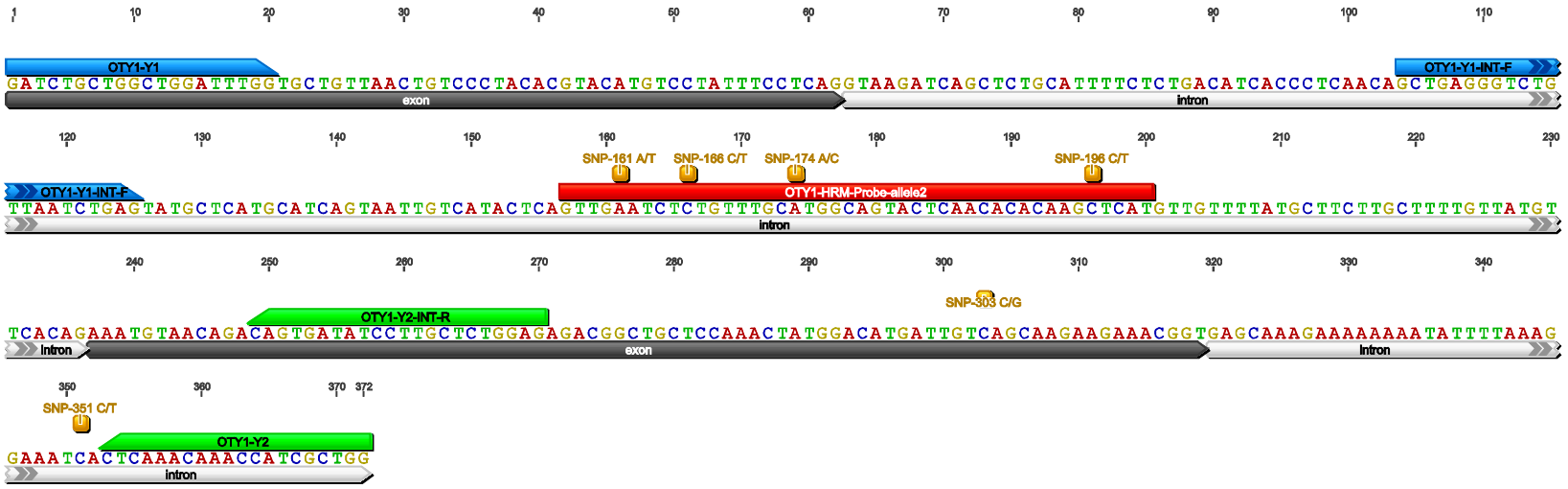
A-4. Sequence segment of the Act2α locus in the alpha skeletal actin gene with associated forward primers (blue), reverse primers (green), SNPs (orange), the Hpy8I restriction site and with the larger fragment (red) and smaller fragment (pink), exons (dark grey), and intron (light grey).



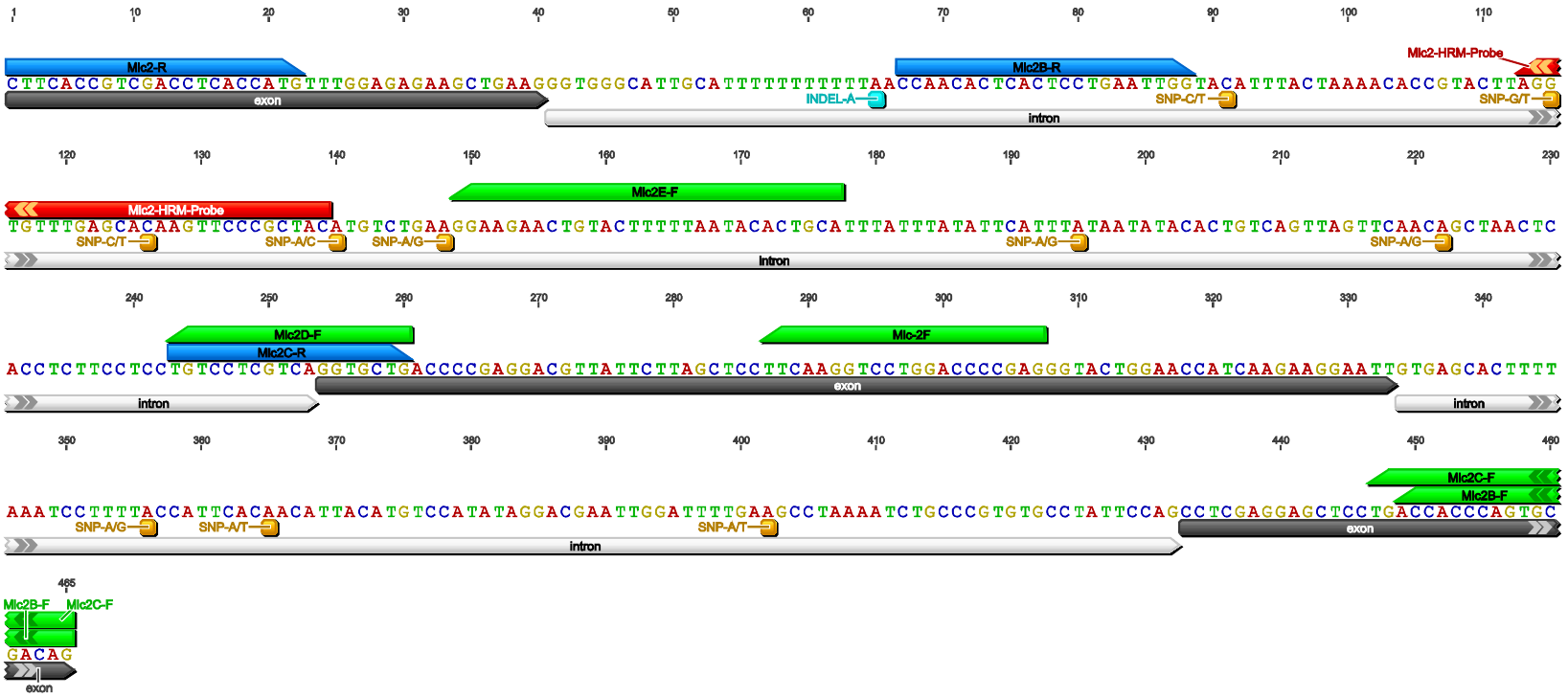
A-5. Sequence segment of the ANT locus in the adenine nucleotide translocator protein with associated forward primers (blue), reverse primers (green), unlabeled probe (red), SNPs (orange), indels (light blue), exons (dark grey), and intron (light grey).



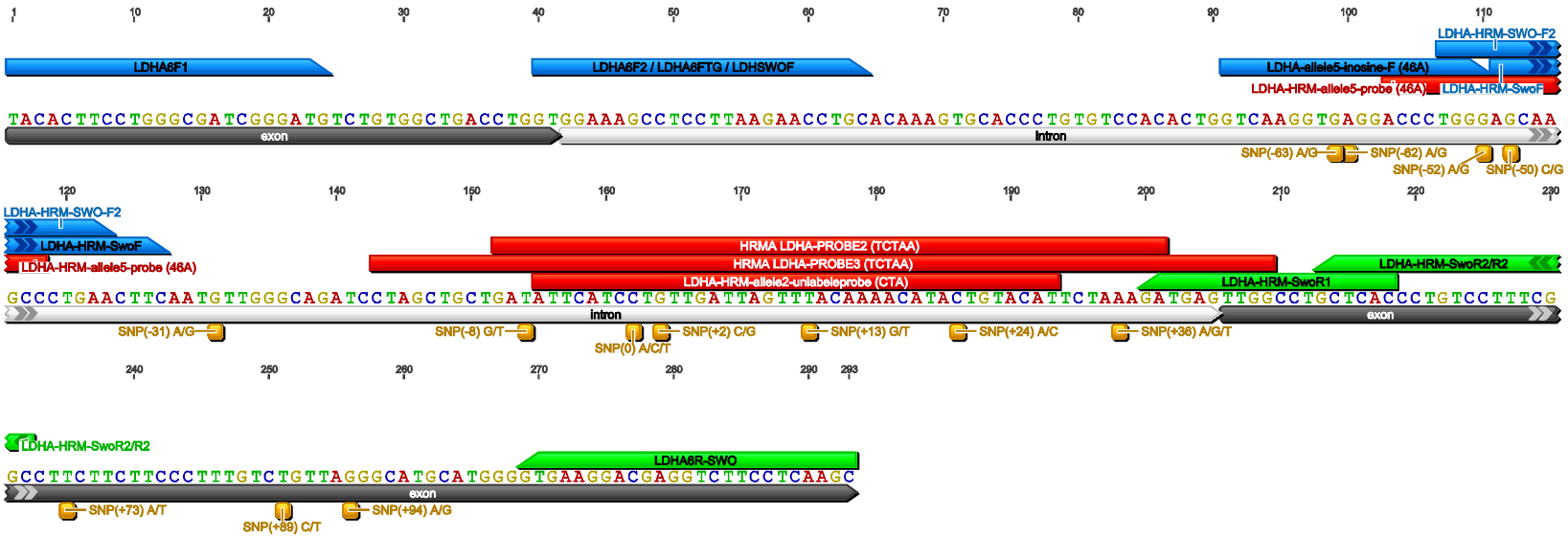
A-6. Sequence segment of the CaM locus in the calmodulin gene with associated forward primers (blue), reverse primers (green), SNP (orange), exons (dark grey), and intron (light grey).



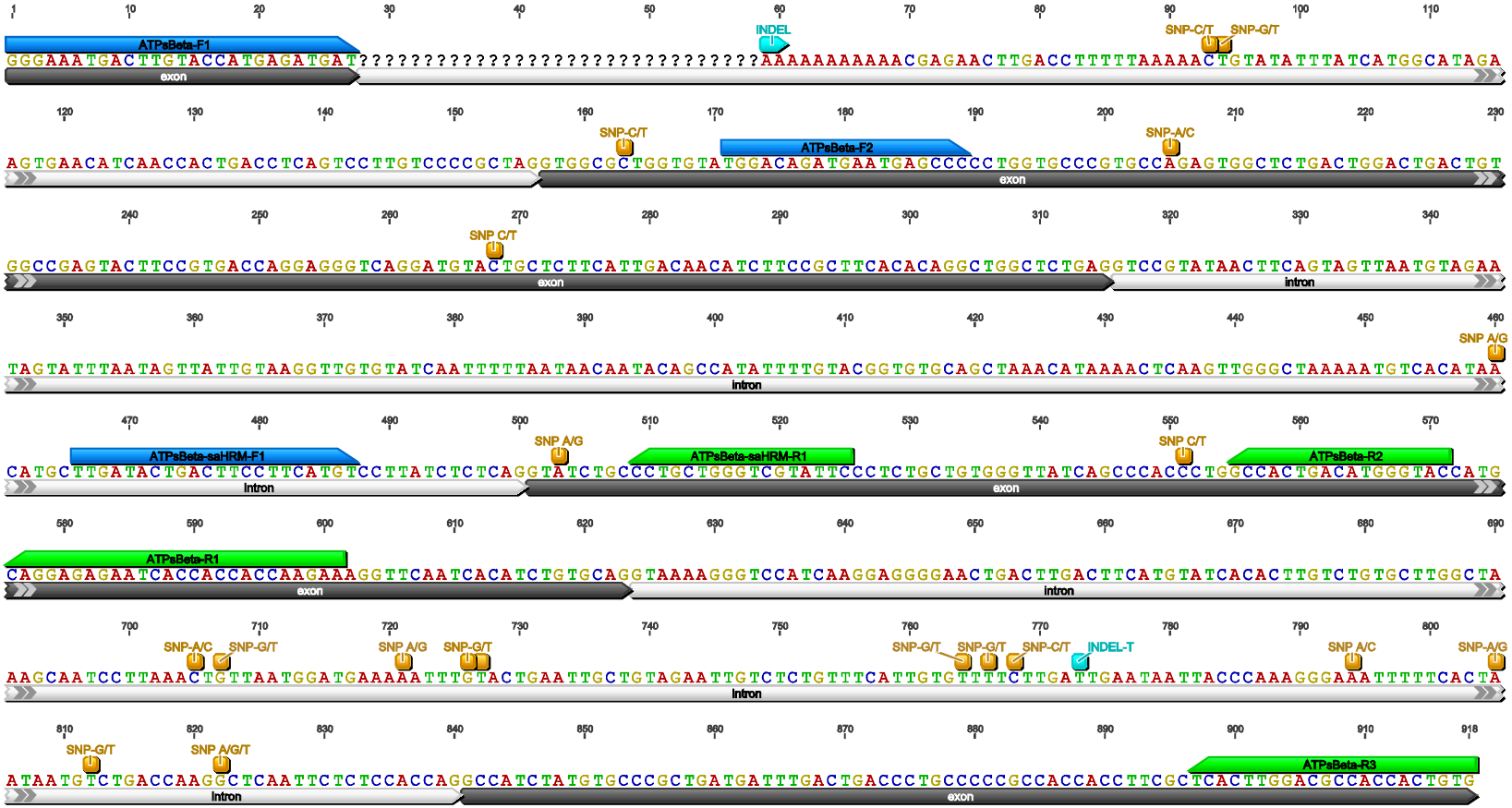
A-7. Sequence segment of the GpHr locus in the golgi pH regulator gene with associated forward primers (blue), reverse primers (green), unlabeled probe (red), SNPs (orange), exons (dark grey), and introns (light grey).



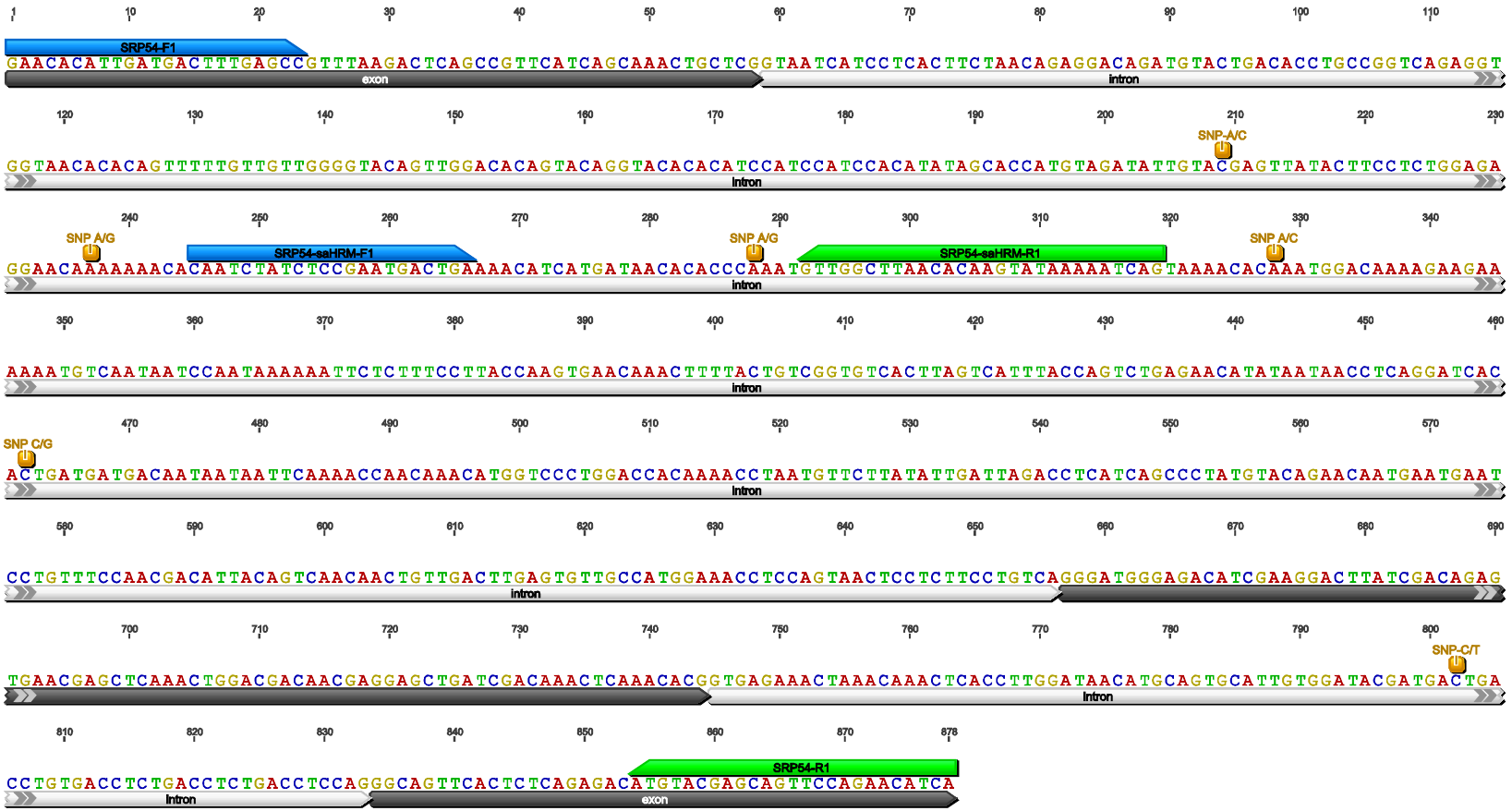
A-8. Sequence segment of the Mlc2 locus in the myosin light chain gene with associated forward primers (blue), reverse primers (green), unlabeled probe (red), SNPs (orange), indels (light blue) exons (dark grey), and introns (light grey).



A-9. Sequence segment of the ldhA locus in the lactate dehydrogenase gene with associated forward primers (blue), reverse primers (green), unlabeled probes (red), SNPs (orange), exons (dark grey), and intron (light grey).



A-10. Sequence segment of the ATPsβ locus in the ATP synthase gene beta-subunit with associated forward primers (blue), reverse primers (green), SNPs (orange), indels (light blue), exons (dark grey), and introns (light grey).



A-11. Sequence segment of the SRP54 locus in the signal recognition particle 54 gene with associated forward primers (blue), reverse primers (green), SNPs (orange), exons (dark grey), and introns (light grey).

A-12. Table of Chapter IV locus allele frequencies for 18 localities of swordfish.

Locus	Allele/n	Locality																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
ARP	**N**	52	49	42	48	17	20	51	83	59	20	37	56	42	45	28	35	44	46
	1	0.337	0.398	0.381	0.333	0.294	0.425	0.324	0.259	0.203	0.400	0.351	0.464	0.393	0.389	0.500	0.371	0.386	0.380
	2	0.663	0.602	0.619	0.667	0.706	0.575	0.676	0.741	0.797	0.600	0.649	0.536	0.607	0.611	0.500	0.629	0.614	0.620
ALDB	**N**	52	49	42	48	17	20	51	83	59	20	37	56	42	45	28	35	44	46
	184	0.106	0.082	0.024	0.083	0.029	0.050	0.098	0.157	0.280	0.050	0.027	0.071	0.095	0.056	0.161	0.071	0.080	0.054
	194	0.019	0.000	0.024	0.000	0.059	0.000	0.010	0.006	0.000	0.025	0.014	0.009	0.000	0.022	0.000	0.000	0.034	0.022
	198	0.837	0.888	0.881	0.885	0.912	0.925	0.863	0.825	0.720	0.900	0.946	0.884	0.869	0.889	0.804	0.871	0.807	0.902
	200	0.000	0.010	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000
	202	0.038	0.020	0.071	0.021	0.000	0.000	0.029	0.012	0.000	0.025	0.014	0.009	0.036	0.011	0.036	0.057	0.045	0.022
	206	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.022	0.000	0.000	0.023	0.000
	208	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000
GpHR	**N**	52	49	42	48	17	20	51	83	59	20	37	56	42	45	28	35	44	46
	1	0.125	0.163	0.107	0.104	0.029	0.000	0.039	0.193	0.119	0.050	0.041	0.054	0.036	0.067	0.036	0.071	0.080	0.043
	2	0.606	0.663	0.667	0.677	0.735	0.800	0.686	0.669	0.669	0.675	0.595	0.714	0.643	0.622	0.589	0.700	0.625	0.707
	3	0.163	0.143	0.119	0.094	0.059	0.125	0.167	0.108	0.178	0.175	0.230	0.107	0.190	0.133	0.143	0.086	0.227	0.141
	4	0.096	0.020	0.095	0.083	0.176	0.075	0.078	0.024	0.034	0.075	0.095	0.107	0.119	0.122	0.179	0.071	0.023	0.065
	5	0.010	0.010	0.012	0.042	0.000	0.000	0.029	0.006	0.000	0.025	0.041	0.018	0.012	0.056	0.054	0.071	0.045	0.043
Act2α	**N**	52	49	42	48	17	20	51	83	59	20	37	56	42	45	28	35	44	46
	1	0.615	0.500	0.643	0.708	0.706	0.850	0.657	0.801	0.805	0.750	0.811	0.598	0.774	0.678	0.786	0.700	0.727	0.761
	2	0.385	0.500	0.357	0.292	0.294	0.150	0.343	0.199	0.195	0.250	0.189	0.402	0.226	0.322	0.214	0.300	0.273	0.239
ATPsβ	**N**	52	49	42	48	17	20	51	83	59	20	37	56	42	45	28	35	44	46
	1	0.346	0.439	0.310	0.354	0.265	0.225	0.461	0.657	0.559	0.450	0.405	0.509	0.357	0.311	0.357	0.329	0.352	0.326
	2	0.654	0.561	0.690	0.646	0.735	0.775	0.539	0.343	0.441	0.550	0.595	0.491	0.643	0.689	0.643	0.671	0.648	0.674
CaM	**N**	52	49	42	48	17	20	51	83	59	20	37	56	42	45	28	35	44	46
	1	0.462	0.541	0.524	0.479	0.529	0.600	0.608	0.464	0.398	0.675	0.716	0.884	0.905	0.922	0.911	0.900	0.920	0.891
	2	0.538	0.459	0.476	0.521	0.471	0.400	0.392	0.536	0.602	0.325	0.284	0.116	0.095	0.078	0.089	0.100	0.080	0.109
Mlc2	**N**	52	49	42	48	17	20	51	83	59	20	37	56	42	45	28	35	44	46
	1	0.885	0.806	0.750	0.740	0.824	0.775	0.775	0.512	0.500	0.750	0.770	0.741	0.798	0.800	0.839	0.686	0.795	0.772
	2	0.106	0.173	0.238	0.229	0.176	0.225	0.206	0.482	0.500	0.200	0.216	0.205	0.155	0.144	0.161	0.271	0.182	0.185
	3	0.010	0.020	0.012	0.031	0.000	0.000	0.020	0.006	0.000	0.050	0.014	0.054	0.048	0.056	0.000	0.043	0.023	0.043
SRP54	**N**	52	49	42	48	17	20	51	83	59	20	37	56	42	45	28	35	44	46
	1	0.798	0.765	0.845	0.792	0.824	0.875	0.784	0.910	0.975	0.900	0.824	0.813	0.774	0.833	0.786	0.743	0.716	0.804
	2	0.202	0.235	0.155	0.208	0.176	0.125	0.216	0.090	0.025	0.100	0.176	0.188	0.226	0.167	0.214	0.257	0.284	0.196
LDHA	**N**	52	49	42	48	17	20	51	83	59	20	37	56	42	45	28	35	44	46
	1	0.683	0.673	0.690	0.656	0.647	0.600	0.578	0.512	0.525	0.600	0.649	0.580	0.619	0.611	0.554	0.543	0.670	0.598
	2	0.029	0.051	0.036	0.042	0.000	0.100	0.049	0.012	0.017	0.025	0.041	0.054	0.024	0.089	0.071	0.071	0.045	0.043
	3	0.288	0.276	0.274	0.302	0.353	0.300	0.373	0.476	0.458	0.375	0.311	0.366	0.357	0.300	0.375	0.386	0.284	0.359
ANT	**N**	52	49	42	48	17	20	51	83	59	20	37	56	42	45	28	35	44	46
	1	0.577	0.622	0.679	0.604	0.794	0.625	0.627	0.560	0.492	0.675	0.595	0.723	0.702	0.589	0.625	0.700	0.625	0.598
	2	0.038	0.020	0.107	0.063	0.000	0.000	0.010	0.006	0.000	0.000	0.027	0.027	0.036	0.011	0.018	0.029	0.000	0.011
	3	0.058	0.041	0.060	0.021	0.059	0.050	0.020	0.030	0.042	0.025	0.041	0.036	0.036	0.067	0.054	0.029	0.023	0.043
	4	0.019	0.020	0.000	0.010	0.029	0.050	0.020	0.012	0.000	0.025	0.027	0.018	0.012	0.011	0.018	0.000	0.011	0.022
	5	0.308	0.296	0.143	0.292	0.088	0.275	0.324	0.392	0.466	0.275	0.297	0.188	0.214	0.311	0.286	0.243	0.341	0.326
	6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.009	0.000	0.000	0.000	0.000	0.000	0.000
	7	0.000	0.000	0.012	0.010	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000

A-13. Table of average population posterior probability membership ($\overline{Q}$) of 18 localities in the Atlantic and Mediterranean inferred in STRUCTURE.

Locality	SA	MED	NWA	n
1	0.046	0.0221	0.9319	52
2	0.0568	0.0258	0.9174	49
3	0.0633	0.0032	0.9335	42
4	0.0411	0.0264	0.9325	48
5	0.0742	0.0042	0.9216	16
6	0.4892	0.0651	0.4457	21
7	0.3564	0.097	0.5466	51
8	0.0653	0.8481	0.0865	83
9	0.008	0.9777	0.0144	59
10	0.5232	0.0226	0.4541	20
11	0.5679	0.016	0.4162	36
12	0.9582	0.0028	0.039	57
13	0.9826	0.0031	0.0144	42
14	0.9868	0.0014	0.0118	45
15	0.9818	0.0023	0.0159	28
16	0.9712	0.0033	0.0254	35
17	0.979	0.0014	0.0196	44
18	0.9776	0.0036	0.0188	46

A-14. Table of individual posterior probability membership ($\overline{Q}$) of 774 Atlantic and Mediterranean swordfish inferred in STRUCTURE v2.3.

	Sample	% missing data	Locality		SA	MED	NA
1	Xgla5835	(0)	1	:	0.1544	0.001	0.8446
2	Xgla5837	(0)	1	:	0.0074	0.0275	0.9651
3	Xgla5839	(0)	1	:	0.0151	0.3165	0.6684
4	Xgla5840	(0)	1	:	0.0049	0.0058	0.9893
5	Xgla5841	(0)	1	:	0.0294	0.071	0.8996
6	Xgla5842	(0)	1	:	0.0152	0	0.9848
7	Xgla5843	(0)	1	:	0.0018	0.007	0.9912
8	Xgla5844	(0)	1	:	0.0059	0.0058	0.9883
9	Xgla5845	(0)	1	:	0.0021	0.0012	0.9967
10	Xgla5846	(0)	1	:	0.0064	0.0034	0.9902
11	Xgla5847	(0)	1	:	0.148	0	0.852
12	Xgla5848	(0)	1	:	0.0225	0.0014	0.9761
13	Xgla5849	(0)	1	:	0.002	0	0.998
14	Xgla5850	(0)	1	:	0.004	0.0035	0.9925
15	Xgla5851	(0)	1	:	0.0697	0	0.9303
16	Xgla5852	(0)	1	:	0.0044	0.0027	0.993
17	Xgla5853	(0)	1	:	0.0014	0.0072	0.9914
18	Xgla5855	(0)	1	:	0.0027	0.0107	0.9866
19	Xgla7344	(0)	1	:	0.0053	0.0066	0.9882
20	Xgla7345	(0)	1	:	0.0301	0	0.9699
21	Xgla7346	(0)	1	:	0.1329	0.0029	0.8642
22	Xgla7348	(0)	1	:	0.0264	0.0086	0.965
23	Xgla7349	(0)	1	:	0.004	0.0014	0.9946
24	Xgla7350	(0)	1	:	0.1552	0.1635	0.6813
25	Xgla7351	(0)	1	:	0.0309	0	0.9691
26	Xgla7352	(0)	1	:	0.0038	0.0045	0.9917
27	Xgla7356	(0)	1	:	0.0156	0.0112	0.9731
28	Xgla7357	(0)	1	:	0.0579	0.1064	0.8357
29	Xgla7358	(0)	1	:	0.0192	0.0025	0.9782
30	Xgla7360	(0)	1	:	0.0019	0.0179	0.9802
31	Xgla7362	(0)	1	:	0.0293	0.0026	0.9681
32	Xgla7365	(0)	1	:	0.0082	0.0008	0.991
33	Xgla7366	(0)	1	:	0.1113	0	0.8887
34	Xgla7368	(0)	1	:	0.001	0	0.999
35	Xgla7369	(0)	1	:	0.0249	0.0029	0.9723
36	Xgla7374	(0)	1	:	0.1274	0.1987	0.6739
37	Xgla7376	(0)	1	:	0.0255	0.0126	0.9619
38	Xgla7377	(0)	1	:	0.0639	0.0024	0.9338
39	Xgla7378	(0)	1	:	0.1552	0.0053	0.8395
40	Xgla7379	(0)	1	:	0.133	0.0028	0.8642
41	Xgla7407	(0)	1	:	0.0606	0.0002	0.9392
42	Xgla7429	(0)	1	:	0.0084	0.0181	0.9735
43	Xgla7436	(0)	1	:	0.2744	0	0.7256
44	Xgla7437	(0)	1	:	0.0045	0.0058	0.9897
45	Xgla7440	(0)	1	:	0.1263	0.0108	0.8629
46	Xgla7443	(0)	1	:	0.1608	0.0026	0.8366
47	Xgla7445	(0)	1	:	0.0013	0.0036	0.9951
48	Xgla7446	(0)	1	:	0.02	0.0304	0.9495
49	Xgla7448	(0)	1	:	0.0065	0.0028	0.9907
50	Xgla7449	(0)	1	:	0.0313	0.0534	0.9153
51	Xgla7452	(0)	1	:	0.0369	0	0.9631
52	Xgla7453	(0)	1	:	0.0011	0.0043	0.9947
53	Xgla1071	(0)	2	:	0.0167	0	0.9833
54	Xgla1072	(0)	2	:	0.0397	0.0018	0.9585
55	Xgla1073	(0)	2	:	0.0167	0	0.9833
56	Xgla1074	(0)	2	:	0.1521	0.001	0.8469
57	Xgla1075	(0)	2	:	0.0295	0.0268	0.9437
58	Xgla1076	(0)	2	:	0.2443	0	0.7556
59	Xgla1077	(0)	2	:	0.0034	0	0.9966
60	Xgla1078	(0)	2	:	0.0209	0.0562	0.9229
61	Xgla1079	(0)	2	:	0.0015	0	0.9985
62	Xgla1080	(0)	2	:	0.1527	0	0.8473

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63	Xgla1081	(0)	2	:	0.0188	0.0299	0.9513
64	Xgla1082	(0)	2	:	0.0776	0.0008	0.9216
65	Xgla1083	(0)	2	:	0.0019	0.0177	0.9804
66	Xgla1084	(0)	2	:	0.0028	0.001	0.9962
67	Xgla1085	(0)	2	:	0.2088	0.0005	0.7906
68	Xgla1086	(0)	2	:	0.0283	0.0017	0.97
69	Xgla1087	(0)	2	:	0.1725	0.0007	0.8268
70	Xgla1088	(0)	2	:	0.035	0.0679	0.8971
71	Xgla1089	(0)	2	:	0.1316	0.005	0.8633
72	Xgla1090	(0)	2	:	0.0505	0.0111	0.9384
73	Xgla1091	(0)	2	:	0.0108	0.016	0.9732
74	Xgla1092	(0)	2	:	0.0033	0.0173	0.9794
75	Xgla1093	(0)	2	:	0.035	0.001	0.964
76	Xgla1094	(0)	2	:	0.014	0.2602	0.7258
77	Xgla1095	(0)	2	:	0.0021	0.057	0.9409
78	Xgla1096	(0)	2	:	0.0259	0.0263	0.9478
79	Xgla1097	(0)	2	:	0.1416	0.001	0.8574
80	Xgla1098	(0)	2	:	0.0112	0.0054	0.9834
81	Xgla1099	(0)	2	:	0.0209	0.0129	0.9662
82	Xgla1100	(0)	2	:	0.0045	0	0.9955
83	Xgla1101	(0)	2	:	0.1685	0.0021	0.8294
84	Xgla1102	(0)	2	:	0.0045	0.1792	0.8163
85	Xgla1103	(0)	2	:	0.03	0.0144	0.9555
86	Xgla1104	(0)	2	:	0.1947	0.024	0.7812
87	Xgla1105	(0)	2	:	0.0153	0.132	0.8527
88	Xgla1106	(0)	2	:	0.0287	0.0063	0.965
89	Xgla1107	(0)	2	:	0.0376	0.0007	0.9617
90	Xgla1108	(0)	2	:	0.0715	0.0001	0.9285
91	Xgla1109	(0)	2	:	0.0093	0.0104	0.9803
92	Xgla1110	(0)	2	:	0.0483	0.0033	0.9484
93	Xgla1111	(0)	2	:	0.0046	0.0072	0.9881
94	Xgla1113	(0)	2	:	0.0275	0.0126	0.9599
95	Xgla1114	(0)	2	:	0.1748	0	0.8252
96	Xgla1122	(0)	2	:	0.0306	0.1733	0.7961
97	Xgla1123	(0)	2	:	0.0132	0.0154	0.9714
98	Xgla1124	(0)	2	:	0.0018	0.0376	0.9606
99	Xgla1125	(0)	2	:	0.0436	0.0273	0.9291
100	Xgla1126	(0)	2	:	0.0393	0.0024	0.9583
101	Xgla1127	(0)	2	:	0.1584	0	0.8416
102	Xgla1892	(0)	3	:	0.0429	0.0027	0.9544
103	Xgla1895	(0)	3	:	0.1296	0	0.8704
104	Xgla1899	(0)	3	:	0.0347	0.0009	0.9644
105	Xgla1900	(0)	3	:	0.0425	0.0105	0.947
106	Xgla1910	(0)	3	:	0.0174	0.0408	0.9418
107	Xgla1920	(0)	3	:	0.0021	0	0.9979
108	Xgla1929	(0)	3	:	0.0018	0	0.9982
109	Xgla1932	(0)	3	:	0.0104	0	0.9896
110	Xgla1938	(0)	3	:	0.0018	0	0.9982
111	Xgla1939	(0)	3	:	0.0168	0.0014	0.9819
112	Xgla1943	(0)	3	:	0.0021	0.0149	0.983
113	Xgla1947	(0)	3	:	0.0193	0.0015	0.9791
114	Xgla1952	(0)	3	:	0.0253	0.0007	0.974
115	Xgla1962	(0)	3	:	0.0723	0	0.9277
116	Xgla1965	(0)	3	:	0.0943	0.0019	0.9039
117	Xgla1966	(0)	3	:	0.2617	0	0.7383
118	Xgla1969	(0)	3	:	0.0262	0	0.9738
119	Xgla1970	(0)	3	:	0.0942	0.0014	0.9044
120	Xgla1973	(0)	3	:	0.035	0.0202	0.9448
121	Xgla1975	(0)	3	:	0.0244	0.0033	0.9723
122	Xgla1983	(0)	3	:	0.0254	0	0.9746
123	Xgla1987	(0)	3	:	0.0013	0	0.9987
124	Xgla1989	(0)	3	:	0.0209	0.0011	0.978
125	Xgla1993	(0)	3	:	0.001	0.0011	0.9979
126	Xgla1994	(0)	3	:	0.1497	0	0.8503
127	Xgla1997	(0)	3	:	0.2013	0.0033	0.7954
128	Xgla2002	(0)	3	:	0.1595	0.0044	0.8361
129	Xgla2003	(0)	3	:	0.0688	0	0.9313
130	Xgla2004	(0)	3	:	0.1808	0	0.8192
131	Xgla2049	(0)	3	:	0.002	0.0018	0.9962
132	Xgla2064	(0)	3	:	0.1624	0	0.8376
133	Xgla2067	(0)	3	:	0.0281	0	0.9719

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134	Xgla2083	(0)	3	:	0.0307	0.0047	0.9646
135	Xgla2084	(0)	3	:	0.1461	0.0015	0.8523
136	Xgla2093	(0)	3	:	0.0304	0.0018	0.9678
137	Xgla2101	(0)	3	:	0.0027	0	0.9973
138	Xgla2105	(0)	3	:	0.0057	0.0018	0.9925
139	Xgla2111	(0)	3	:	0.0034	0.0017	0.9949
140	Xgla2120	(0)	3	:	0.1903	0.0008	0.8089
141	Xgla2158	(0)	3	:	0.021	0.0009	0.978
142	Xgla2163	(0)	3	:	0.2428	0.0128	0.7445
143	Xgla2171	(0)	3	:	0.0289	0	0.9712
144	Xgla700	(0)	4	:	0.0168	0.001	0.9822
145	Xgla701	(0)	4	:	0.026	0.0018	0.9722
146	Xgla702	(0)	4	:	0.0048	0.0001	0.9951
147	Xgla703	(0)	4	:	0.0125	0	0.9875
148	Xgla704	(0)	4	:	0.123	0.0133	0.8637
149	Xgla705	(0)	4	:	0.003	0.2522	0.7448
150	Xgla706	(0)	4	:	0.0086	0.002	0.9894
151	Xgla707	(0)	4	:	0.016	0.0468	0.9371
152	Xgla708	(0)	4	:	0.0919	0.0153	0.8928
153	Xgla709	(0)	4	:	0.0702	0.0381	0.8917
154	Xgla733	(0)	4	:	0.0013	0	0.9987
155	Xgla734	(0)	4	:	0.0678	0	0.9322
156	Xgla735	(0)	4	:	0.1245	0.0051	0.8704
157	Xgla736	(0)	4	:	0.0405	0.0059	0.9536
158	Xgla737	(0)	4	:	0.1312	0.0058	0.863
159	Xgla746	(0)	4	:	0.0473	0.0011	0.9516
160	Xgla747	(0)	4	:	0.0329	0.0205	0.9465
161	Xgla749	(0)	4	:	0.062	0.0965	0.8416
162	Xgla750	(0)	4	:	0.1191	0.0005	0.8804
163	Xgla751	(0)	4	:	0.0335	0	0.9665
164	Xgla752	(0)	4	:	0.01	0.0912	0.8987
165	Xgla753	(0)	4	:	0.0165	0.0178	0.9658
166	Xgla755	(0)	4	:	0.1235	0.0031	0.8733
167	Xgla1246	(0)	4	:	0.0064	0.0384	0.9552
168	Xgla1247	(0)	4	:	0.0316	0.0054	0.963
169	Xgla1248	(0)	4	:	0.018	0.004	0.978
170	Xgla1249	(0)	4	:	0.1412	0.0023	0.8565
171	Xgla1250	(0)	4	:	0.0268	0.0118	0.9614
172	Xgla1251	(0)	4	:	0.147	0.0028	0.8502
173	Xgla1252	(0)	4	:	0.1894	0.008	0.8026
174	Xgla1253	(0)	4	:	0.0033	0.0025	0.9942
175	Xgla1254	(0)	4	:	0.0229	0.005	0.9721
176	Xgla1255	(0)	4	:	0.0222	0.151	0.8269
177	Xgla1256	(0)	4	:	0.0052	0.0035	0.9914
178	Xgla1257	(0)	4	:	0.0055	0.0003	0.9942
179	Xgla1258	(0)	4	:	0.0011	0.0228	0.9761
180	Xgla1259	(0)	4	:	0.0301	0	0.9699
181	Xgla1260	(0)	4	:	0.0049	0	0.9951
182	Xgla1261	(0)	4	:	0.0026	0.083	0.9144
183	Xgla1262	(0)	4	:	0.043	0.0709	0.8861
184	Xgla1263	(0)	4	:	0.0193	0.0025	0.9783
185	Xgla1264	(0)	4	:	0.0032	0.2166	0.7802
186	Xgla1265	(0)	4	:	0.0243	0	0.9757
187	Xgla1266	(0)	4	:	0.0215	0.0096	0.9689
188	Xgla1267	(0)	4	:	0.0008	0.0028	0.9963
189	Xgla1268	(0)	4	:	0.0136	0.001	0.9854
190	Xgla1269	(0)	4	:	0.0028	0.001	0.9962
191	Xgla6380	(0)	4	:	0.0039	0.0047	0.9913
192	Xgla6367	(0)	5	:	0.081	0	0.919
193	Xgla6368	(0)	5	:	0.0071	0.002	0.9909
194	Xgla6374	(0)	5	:	0.0348	0.0251	0.9401
195	Xgla6376	(0)	5	:	0.0114	0.0058	0.9828
196	Xgla6377	(0)	5	:	0.0533	0.0004	0.9462
197	Xgla6379	(0)	5	:	0.2381	0.007	0.7549
198	Xgla6382	(0)	5	:	0.0524	0.0017	0.9458
199	Xgla6386	(0)	5	:	0.007	0.0192	0.9738
200	Xgla6388	(0)	5	:	0.0061	0	0.9939
201	Xgla6391	(0)	5	:	0.028	0	0.972
202	Xgla7610	(0)	5	:	0.0542	0	0.9458
203	Xgla7611	(0)	5	:	0.0396	0.001	0.9594
204	Xgla7612	(0)	5	:	0.2463	0	0.7537

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205	Xgla7613	(0)	5	:	0.045	0.001	0.954
206	Xgla7614	(0)	5	:	0.2377	0.0043	0.7581
207	Xgla7714	(0)	5	:	0.0424	0.0006	0.9569
208	Xgla268	(0)	6	:	0.7708	0.0073	0.2219
209	Xgla269	(0)	6	:	0.793	0	0.207
210	Xgla270	(0)	6	:	0.1127	0.0461	0.8412
211	Xgla274	(0)	6	:	0.3973	0.0147	0.588
212	Xgla6363	(0)	6	:	0.9488	0	0.0512
213	Xgla6364	(0)	6	:	0.0805	0.1993	0.7201
214	Xgla6365	(0)	6	:	0.2123	0.3783	0.4093
215	Xgla6369	(0)	6	:	0.837	0.0011	0.1619
216	Xgla6370	(0)	6	:	0.0525	0.2008	0.7467
217	Xgla6371	(0)	6	:	0.7277	0.0003	0.272
218	Xgla6372	(0)	6	:	0.3707	0.0054	0.6239
219	Xgla6373	(0)	6	:	0.7721	0.0036	0.2243
220	Xgla6375	(0)	6	:	0.7781	0.0069	0.215
221	Xgla6378	(0)	6	:	0.0828	0.1592	0.758
222	Xgla6381	(0)	6	:	0.6908	0.0053	0.3039
223	Xgla6383	(0)	6	:	0.818	0.0009	0.181
224	Xgla6384	(0)	6	:	0.3169	0.0842	0.5989
225	Xgla6387	(0)	6	:	0.306	0.207	0.487
226	Xgla6389	(0)	6	:	0.4673	0.0085	0.5242
227	Xgla6390	(0)	6	:	0.3732	0.0038	0.623
228	Xgla6392	(0)	6	:	0.3649	0.0358	0.5993
229	Xgla260	(0)	7	:	0.6166	0.0022	0.3812
230	Xgla262	(0)	7	:	0.6734	0.0048	0.3217
231	Xgla264	(0)	7	:	0.6592	0.0009	0.3399
232	Xgla266	(0)	7	:	0.229	0.0103	0.7607
233	Xgla267	(0)	7	:	0.7049	0.0818	0.2133
234	Xgla271	(0)	7	:	0.0494	0.0559	0.8947
235	Xgla272	(0)	7	:	0.294	0.0914	0.6146
236	Xgla273	(0)	7	:	0.7552	0.0045	0.2403
237	Xgla7609	(0)	7	:	0.2173	0.0456	0.7371
238	Xgla7615	(0)	7	:	0.6277	0.0682	0.3041
239	Xgla7616	(0)	7	:	0.2964	0.1344	0.5692
240	Xgla7617	(0)	7	:	0.2738	0	0.7262
241	Xgla7618	(0)	7	:	0.061	0.5172	0.4219
242	Xgla7619	(0)	7	:	0.6554	0.0122	0.3324
243	Xgla7620	(0)	7	:	0.3134	0.0023	0.6844
244	Xgla7621	(0)	7	:	0.0282	0.2079	0.7639
245	Xgla7622	(0)	7	:	0.1916	0	0.8084
246	Xgla7623	(0)	7	:	0.4177	0.1539	0.4284
247	Xgla7624	(0)	7	:	0.0091	0	0.9909
248	Xgla7625	(0)	7	:	0.6005	0	0.3994
249	Xgla7626	(0)	7	:	0.0986	0.0683	0.8331
250	Xgla7627	(0)	7	:	0.5415	0.0069	0.4516
251	Xgla7628	(0)	7	:	0.0082	0.9585	0.0332
252	Xgla7629	(0)	7	:	0.1651	0.0075	0.8274
253	Xgla7630	(0)	7	:	0.1945	0.0434	0.7622
254	Xgla7631	(0)	7	:	0.2233	0.0791	0.6976
255	Xgla7632	(0)	7	:	0.0741	0.0235	0.9024
256	Xgla7633	(0)	7	:	0.6785	0.0043	0.3171
257	Xgla7667	(0)	7	:	0.049	0.017	0.9341
258	Xgla7668	(0)	7	:	0.6049	0.0201	0.375
259	Xgla7669	(0)	7	:	0.0908	0.6576	0.2516
260	Xgla7670	(0)	7	:	0.0538	0.3437	0.6025
261	Xgla7671	(0)	7	:	0.6765	0.0034	0.3201
262	Xgla7672	(0)	7	:	0.2471	0.0021	0.7508
263	Xgla7673	(0)	7	:	0.2148	0.0112	0.774
264	Xgla7674	(0)	7	:	0.0347	0.1342	0.8311
265	Xgla7675	(0)	7	:	0.2504	0.0054	0.7441
266	Xgla7676	(0)	7	:	0.1522	0.0207	0.8271
267	Xgla7677	(0)	7	:	0.042	0.0556	0.9024
268	Xgla7678	(0)	7	:	0.5024	0.3054	0.1922
269	Xgla7679	(0)	7	:	0.7146	0.0056	0.2798
270	Xgla7680	(0)	7	:	0.0367	0.2263	0.737
271	Xgla7681	(0)	7	:	0.5878	0	0.4122
272	Xgla7682	(0)	7	:	0.57	0.0075	0.4225
273	Xgla7683	(0)	7	:	0.211	0.001	0.788
274	Xgla7684	(0)	7	:	0.7483	0.0021	0.2496
275	Xgla7716	(0)	7	:	0.6636	0.0053	0.3311

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276	Xgla7717	(0)	7	:	0.7458	0.0064	0.2479
277	Xgla7718	(0)	7	:	0.6972	0.0114	0.2914
278	Xgla7813	(0)	7	:	0.6036	0.1192	0.2772
279	Xgla7815	(0)	7	:	0.0179	0.4007	0.5814
280	Xgla7582	(0)	8	:	0.0093	0.9468	0.044
281	Xgla7583	(0)	8	:	0.0295	0.9285	0.042
282	Xgla7584	(0)	8	:	0.0767	0.7323	0.191
283	Xgla7586	(0)	8	:	0.0025	0.9888	0.0087
284	Xgla7587	(0)	8	:	0.0001	0.998	0.002
285	Xgla7588	(0)	8	:	0.1558	0.8064	0.0378
286	Xgla7589	(0)	8	:	0.0161	0.9497	0.0342
287	Xgla7590	(0)	8	:	0	0.999	0.001
288	Xgla7591	(0)	8	:	0	1	0
289	Xgla7592	(0)	8	:	0.0001	0.9989	0.001
290	Xgla7593	(0)	8	:	0.0021	0.9918	0.0062
291	Xgla7595	(0)	8	:	0.0091	0.7184	0.2725
292	Xgla7596	(0)	8	:	0	0.9943	0.0057
293	Xgla7597	(0)	8	:	0.013	0.9464	0.0406
294	Xgla7599	(0)	8	:	0.0126	0.7139	0.2735
295	Xgla7600	(0)	8	:	0	0.999	0.001
296	Xgla7601	(0)	8	:	0.0011	0.9952	0.0038
297	Xgla7777	(0)	8	:	0.005	0.9656	0.0294
298	Xgla7778	(0)	8	:	0.0065	0.9707	0.0228
299	Xgla7779	(0)	8	:	0.0378	0.9069	0.0554
300	Xgla7781	(0)	8	:	0.001	0.9934	0.0056
301	Xgla7782	(0)	8	:	0.001	0.9962	0.0028
302	Xgla7783	(0)	8	:	0.0084	0.7898	0.2018
303	Xgla7784	(0)	8	:	0	0.9911	0.0089
304	Xgla7785	(0)	8	:	0.0405	0.9441	0.0154
305	Xgla7786	(0)	8	:	0.0304	0.772	0.1976
306	Xgla7787	(0)	8	:	0	1	0
307	Xgla7788	(0)	8	:	0.003	0.9763	0.0207
308	Xgla7790	(0)	8	:	0.0068	0.9715	0.0217
309	Xgla7791	(0)	8	:	0.0031	0.996	0.0009
310	Xgla7792	(0)	8	:	0.0021	0.992	0.0059
311	Xgla7793	(0)	8	:	0	0.998	0.002
312	Xgla7794	(0)	8	:	0.2901	0.6225	0.0874
313	Xgla7795	(0)	8	:	0.0001	0.9858	0.014
314	Xgla7796	(0)	8	:	0.0585	0.9106	0.0308
315	Xgla7797	(0)	8	:	0.5723	0.0569	0.3708
316	Xgla7799	(0)	8	:	0.0056	0.75	0.2444
317	Xgla7800	(0)	8	:	0.3251	0.5266	0.1483
318	Xgla7801	(0)	8	:	0.0009	0.9657	0.0334
319	Xgla7803	(0)	8	:	0.0033	0.9758	0.0209
320	Xgla7804	(0)	8	:	0	0.999	0.001
321	Xgla7805	(0)	8	:	0.3586	0.0033	0.6382
322	Xgla7806	(0)	8	:	0.0002	0.9893	0.0106
323	Xgla7808	(0)	8	:	0.0479	0.6932	0.2589
324	Xgla7810	(0)	8	:	0.0004	0.9917	0.0078
325	Xgla7811	(0)	8	:	0.2473	0.6856	0.0671
326	Xgla7812	(0)	8	:	0.006	0.8315	0.1625
327	Xgla5774	(0)	8	:	0.002	0.9813	0.0167
328	Xgla5795	(0)	8	:	0	0.999	0.001
329	Xgla5796	(0)	8	:	0.0002	0.9906	0.0092
330	Xgla5797	(0)	8	:	0.001	0.9915	0.0075
331	Xgla5798	(0)	8	:	0.0261	0.9665	0.0074
332	Xgla5799	(0)	8	:	0.0174	0.9265	0.0561
333	Xgla5800	(0)	8	:	0.001	0.9916	0.0074
334	Xgla5801	(0)	8	:	0.0757	0.8596	0.0647
335	Xgla5802	(0)	8	:	0.0041	0.8611	0.1348
336	Xgla5803	(0)	8	:	0.0196	0.9727	0.0077
337	Xgla5804	(0)	8	:	0.0763	0.7923	0.1314
338	Xgla5805	(0)	8	:	0.0114	0.9408	0.0478
339	Xgla5807	(0)	8	:	0	1	0.0001
340	Xgla5808	(0)	8	:	0.0061	0.992	0.0019
341	Xgla5809	(0)	8	:	0.0128	0.9834	0.0038
342	Xgla5810	(0)	8	:	0.0099	0.9349	0.0552
343	Xgla5811	(0)	8	:	0	0.9999	0.0001
344	Xgla5812	(0)	8	:	0	0.999	0.001
345	Xgla5813	(0)	8	:	0.0675	0.8997	0.0328
346	Xgla5814	(0)	8	:	0.0054	0.8524	0.1421

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347	Xgla7739	(0)	8	:	0.001	0.9687	0.0303
348	Xgla7740	(0)	8	:	0.0058	0.9905	0.0037
349	Xgla7741	(0)	8	:	0.0993	0.5221	0.3786
350	Xgla7742	(0)	8	:	0.0002	0.9652	0.0346
351	Xgla7743	(0)	8	:	0.2746	0.6383	0.0871
352	Xgla7745	(0)	8	:	0.1593	0.7564	0.0843
353	Xgla7746	(0)	8	:	0.0032	0.9306	0.0662
354	Xgla7747	(0)	8	:	0.005	0.9821	0.0129
355	Xgla7748	(0)	8	:	0	0.999	0.001
356	Xgla7749	(0)	8	:	0.2393	0.0145	0.7462
357	Xgla7750	(0)	8	:	0.6714	0.0232	0.3054
358	Xgla7751	(0)	8	:	0.7662	0	0.2338
359	Xgla7752	(0)	8	:	0.4374	0.0488	0.5138
360	Xgla7753	(0)	8	:	0.0001	0.9979	0.002
361	Xgla7754	(0)	8	:	0.0058	0.7189	0.2753
362	Xgla7755	(0)	8	:	0.015	0.9557	0.0293
363	Xgla249	(0)	9	:	0.115	0.8305	0.0545
364	Xgla277	(0)	9	:	0	1	0
365	Xgla280	(0)	9	:	0	1	0
366	Xgla281	(0)	9	:	0	0.988	0.012
367	Xgla282	(0)	9	:	0.0008	0.9975	0.0017
368	Xgla288	(0)	9	:	0.001	0.9909	0.0081
369	Xgla290	(0)	9	:	0	0.9982	0.0018
370	Xgla315	(0)	9	:	0.0009	0.9844	0.0146
371	Xgla317	(0)	9	:	0.0432	0.7606	0.1962
372	Xgla318	(0)	9	:	0	0.9989	0.0011
373	Xgla319	(0)	9	:	0	0.9992	0.0008
374	Xgla321	(0)	9	:	0.0222	0.9382	0.0396
375	Xgla322	(0)	9	:	0.0101	0.9548	0.0351
376	Xgla323	(0)	9	:	0	0.9981	0.0019
377	Xgla326	(0)	9	:	0.0001	0.9968	0.0031
378	Xgla327	(0)	9	:	0.0044	0.9947	0.0009
379	Xgla329	(0)	9	:	0.0111	0.821	0.1679
380	Xgla330	(0)	9	:	0	0.999	0.001
381	Xgla331	(0)	9	:	0.0079	0.9864	0.0057
382	Xgla332	(0)	9	:	0	0.9987	0.0013
383	Xgla333	(0)	9	:	0	1	0
384	Xgla334	(0)	9	:	0.0038	0.9514	0.0447
385	Xgla335	(0)	9	:	0.002	0.9919	0.0061
386	Xgla336	(0)	9	:	0.0984	0.8198	0.0818
387	Xgla337	(0)	9	:	0	1	0
388	Xgla341	(0)	9	:	0.0007	0.9903	0.009
389	Xgla342	(0)	9	:	0.0009	0.9947	0.0044
390	Xgla343	(0)	9	:	0	1	0
391	Xgla344	(0)	9	:	0.0043	0.9944	0.0013
392	Xgla346	(0)	9	:	0	1	0.0001
393	Xgla347	(0)	9	:	0.001	0.9949	0.0041
394	Xgla348	(0)	9	:	0.002	0.993	0.005
395	Xgla349	(0)	9	:	0	0.995	0.005
396	Xgla350	(0)	9	:	0	0.9981	0.0019
397	Xgla352	(0)	9	:	0	0.9995	0.0006
398	Xgla353	(0)	9	:	0.1019	0.8592	0.0389
399	Xgla355	(0)	9	:	0.0108	0.9852	0.004
400	Xgla356	(0)	9	:	0.003	0.9882	0.0089
401	Xgla360	(0)	9	:	0.001	0.9915	0.0075
402	Xgla361	(0)	9	:	0.0024	0.9934	0.0042
403	Xgla367	(0)	9	:	0	0.999	0.001
404	Xgla368	(0)	9	:	0.001	0.9968	0.0022
405	Xgla370	(0)	9	:	0.001	0.995	0.004
406	Xgla5757	(0)	9	:	0.0027	0.9963	0.001
407	Xgla5759	(0)	9	:	0.0017	0.9604	0.0379
408	Xgla5760	(0)	9	:	0	0.9993	0.0007
409	Xgla5761	(0)	9	:	0	0.999	0.001
410	Xgla5762	(0)	9	:	0.0018	0.9941	0.0041
411	Xgla5763	(0)	9	:	0.001	0.998	0.001
412	Xgla5764	(0)	9	:	0.0009	0.9981	0.001
413	Xgla5765	(0)	9	:	0	0.9996	0.0004
414	Xgla5766	(0)	9	:	0	0.9952	0.0048
415	Xgla5767	(0)	9	:	0.0113	0.9807	0.008
416	Xgla5768	(0)	9	:	0.001	0.9965	0.0025
417	Xgla5769	(0)	9	:	0	0.9987	0.0013

418	Xgla5770	(0)	9	:	0	1	0
419	Xgla5771	(0)	9	:	0	1	0
420	Xgla5772	(0)	9	:	0	0.999	0.001
421	Xgla5773	(0)	9	:	0	0.9961	0.0039
422	Xgla7756	(0)	10	:	0.4097	0.0011	0.5892
423	Xgla7757	(0)	10	:	0.6563	0.0088	0.3349
424	Xgla7758	(0)	10	:	0.7059	0.0372	0.257
425	Xgla7759	(0)	10	:	0.6569	0	0.3431
426	Xgla7760	(0)	10	:	0.8084	0.0021	0.1894
427	Xgla7761	(0)	10	:	0.3	0.0502	0.6498
428	Xgla7763	(0)	10	:	0.4824	0.0226	0.4949
429	Xgla7764	(0)	10	:	0.3956	0.0603	0.5441
430	Xgla7765	(0)	10	:	0.187	0.004	0.809
431	Xgla7766	(0)	10	:	0.8278	0.001	0.1712
432	Xgla7767	(0)	10	:	0.1323	0.0025	0.8652
433	Xgla7768	(0)	10	:	0.7676	0.0135	0.2189
434	Xgla7769	(0)	10	:	0.3508	0.0017	0.6474
435	Xgla7770	(0)	10	:	0.1323	0.0009	0.8668
436	Xgla7771	(0)	10	:	0.4236	0.0612	0.5152
437	Xgla7772	(0)	10	:	0.3404	0.0019	0.6577
438	Xgla7773	(0)	10	:	0.663	0.1028	0.2342
439	Xgla7774	(0)	10	:	0.7129	0.0754	0.2116
440	Xgla7775	(0)	10	:	0.7676	0.0053	0.2271
441	Xgla7776	(0)	10	:	0.7456	0.0004	0.254
442	Xgla243	(0)	11	:	0.3888	0.0143	0.5968
443	Xgla244	(0)	11	:	0.3726	0.0114	0.616
444	Xgla245	(0)	11	:	0.6959	0.0075	0.2966
445	Xgla246	(0)	11	:	0.8584	0	0.1416
446	Xgla247	(0)	11	:	0.7501	0	0.2498
447	Xgla253	(0)	11	:	0.3565	0.0071	0.6364
448	Xgla254	(0)	11	:	0.4039	0.0061	0.5901
449	Xgla255	(0)	11	:	0.7524	0.0013	0.2462
450	Xgla258	(0)	11	:	0.464	0.0359	0.5001
451	Xgla7602	(0)	11	:	0.8178	0.0066	0.1756
452	Xgla7603	(0)	11	:	0.8179	0.002	0.1801
453	Xgla7604	(0)	11	:	0.2819	0.0056	0.7125
454	Xgla7605	(0)	11	:	0.2245	0.0204	0.7552
455	Xgla7606	(0)	11	:	0.8344	0	0.1656
456	Xgla7607	(0)	11	:	0.8464	0	0.1536
457	Xgla7608	(0)	11	:	0.6713	0.0001	0.3286
458	Xgla7715	(0)	11	:	0.8862	0	0.1138
459	Xgla7719	(0)	11	:	0.6183	0	0.3817
460	Xgla7720	(0)	11	:	0.3228	0.001	0.6762
461	Xgla7721	(0)	11	:	0.2789	0.006	0.7151
462	Xgla7722	(0)	11	:	0.3466	0.0263	0.6271
463	Xgla7723	(0)	11	:	0.3649	0.002	0.6331
464	Xgla7724	(0)	11	:	0.2803	0.0828	0.6369
465	Xgla7725	(0)	11	:	0.8095	0.0018	0.1887
466	Xgla7726	(0)	11	:	0.3667	0.0011	0.6322
467	Xgla7727	(0)	11	:	0.8383	0	0.1617
468	Xgla7728	(0)	11	:	0.9114	0.001	0.0876
469	Xgla7729	(0)	11	:	0.841	0.0003	0.1587
470	Xgla7730	(0)	11	:	0.0738	0.131	0.7952
471	Xgla7731	(0)	11	:	0.4093	0.0455	0.5452
472	Xgla7732	(0)	11	:	0.7325	0.1099	0.1576
473	Xgla7733	(0)	11	:	0.1002	0.0182	0.8815
474	Xgla7734	(0)	11	:	0.865	0.0058	0.1292
475	Xgla7735	(0)	11	:	0.8324	0.013	0.1546
476	Xgla7736	(0)	11	:	0.7846	0.0065	0.2089
477	Xgla7737	(0)	11	:	0.2424	0.0014	0.7562
478	Xgla7738	(0)	12	:	0.9944	0	0.0056
479	Xgla6255	(0)	12	:	0.8727	0.0019	0.1254
480	Xgla6256	(0)	12	:	0.9829	0	0.0171
481	Xgla6257	(0)	12	:	0.9854	0.0009	0.0137
482	Xgla6258	(0)	12	:	0.9758	0	0.0242
483	Xgla6259	(0)	12	:	0.9837	0.001	0.0153
484	Xgla6260	(0)	12	:	0.9986	0	0.0014
485	Xgla6261	(0)	12	:	0.9989	0	0.0011
486	Xgla6263	(0)	12	:	0.9809	0	0.0191
487	Xgla6264	(0)	12	:	0.9628	0	0.0372
488	Xgla6266	(0)	12	:	0.9366	0.0003	0.0631

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489	Xgla6267	(0)	12	:	0.9729	0	0.0271
490	Xgla6268	(0)	12	:	0.9533	0	0.0467
491	Xgla6270	(0)	12	:	0.9873	0	0.0128
492	Xgla6271	(0)	12	:	0.9132	0.0009	0.0858
493	Xgla6272	(0)	12	:	0.9923	0	0.0078
494	Xgla6273	(0)	12	:	0.9854	0.0028	0.0118
495	Xgla6274	(0)	12	:	0.9369	0.0267	0.0364
496	Xgla6275	(0)	12	:	0.963	0.0036	0.0334
497	Xgla6276	(0)	12	:	0.9855	0.0016	0.0129
498	Xgla6277	(0)	12	:	0.9864	0	0.0136
499	Xgla6279	(0)	12	:	0.9888	0	0.0112
500	Xgla6280	(0)	12	:	0.9768	0	0.0232
501	Xgla6281	(0)	12	:	0.9949	0	0.0051
502	Xgla6282	(0)	12	:	0.9702	0.0145	0.0153
503	Xgla6284	(0)	12	:	0.9939	0	0.0061
504	Xgla6285	(0)	12	:	0.9381	0.0004	0.0614
505	Xgla6286	(0)	12	:	0.9835	0.0017	0.0149
506	Xgla6287	(0)	12	:	0.9865	0	0.0135
507	Xgla6288	(0)	12	:	0.9445	0.0012	0.0543
508	Xgla6289	(0)	12	:	0.9813	0.001	0.0177
509	Xgla6290	(0)	12	:	0.9944	0	0.0056
510	Xgla6296	(0)	12	:	0.9951	0	0.0049
511	Xgla6298	(0)	12	:	0.925	0	0.075
512	Xgla6299	(0)	12	:	0.8168	0	0.1832
513	Xgla6300	(0)	12	:	0.9657	0.0058	0.0284
514	Xgla6301	(0)	12	:	0.9836	0	0.0164
515	Xgla6302	(0)	12	:	0.9847	0.0006	0.0146
516	Xgla6303	(0)	12	:	0.9925	0	0.0075
517	Xgla6304	(0)	12	:	0.9845	0	0.0155
518	Xgla6305	(0)	12	:	0.9494	0.0009	0.0497
519	Xgla6306	(0)	12	:	0.8831	0.0007	0.1161
520	Xgla6751	(0)	12	:	0.981	0.0027	0.0163
521	Xgla6752	(0)	12	:	0.8656	0	0.1344
522	Xgla6754	(0)	12	:	0.9774	0	0.0226
523	Xgla6755	(0)	12	:	0.9744	0	0.0256
524	Xgla6756	(0)	12	:	0.9829	0	0.0171
525	Xgla6757	(0)	12	:	0.9818	0.0024	0.0157
526	Xgla6758	(0)	12	:	0.8522	0.0047	0.1431
527	Xgla6759	(0)	12	:	0.876	0.0289	0.0952
528	Xgla6760	(0)	12	:	0.8575	0.0166	0.1259
529	Xgla6761	(0)	12	:	0.9936	0.0001	0.0063
530	Xgla6762	(0)	12	:	0.7966	0.0275	0.1758
531	Xgla6763	(0)	12	:	0.9747	0	0.0253
532	Xgla6764	(0)	12	:	0.9711	0	0.0289
533	Xgla6765	(0)	12	:	0.9758	0.0024	0.0218
534	Xgla6766	(0)	12	:	0.9759	0.0022	0.0219
535	Xgla235	(0)	13	:	0.9562	0.0021	0.0417
536	Xgla236	(0)	13	:	0.9091	0.0106	0.0803
537	Xgla239	(0)	13	:	0.9949	0	0.0051
538	Xgla241	(0)	13	:	0.9907	0.0012	0.0081
539	Xgla4519	(0)	13	:	0.9863	0	0.0137
540	Xgla4520	(0)	13	:	0.9934	0	0.0066
541	Xgla4521	(0)	13	:	0.9505	0.0048	0.0447
542	Xgla4522	(0)	13	:	0.9792	0	0.0208
543	Xgla4523	(0)	13	:	0.9883	0.0019	0.0098
544	Xgla4524	(0)	13	:	0.9921	0.0051	0.0028
545	Xgla4525	(0)	13	:	0.9818	0.0116	0.0066
546	Xgla4526	(0)	13	:	0.9918	0.0011	0.0071
547	Xgla4531	(0)	13	:	0.993	0	0.007
548	Xgla4532	(0)	13	:	0.9822	0	0.0178
549	Xgla4533	(0)	13	:	0.9776	0.0106	0.0117
550	Xgla4534	(0)	13	:	0.9929	0.0008	0.0063
551	Xgla4535	(0)	13	:	0.988	0	0.0121
552	Xgla4537	(0)	13	:	0.9962	0	0.0038
553	Xgla4538	(0)	13	:	0.9639	0.0142	0.0219
554	Xgla4539	(0)	13	:	0.9799	0	0.0201
555	Xgla4540	(0)	13	:	0.9802	0.0011	0.0188
556	Xgla4541	(0)	13	:	0.9906	0.0005	0.0089
557	Xgla4542	(0)	13	:	0.9925	0.0018	0.0057
558	Xgla4543	(0)	13	:	0.9931	0	0.0069
559	Xgla4544	(0)	13	:	0.9828	0.0006	0.0166

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560	Xgla4545	(0)	13	:	0.9933	0	0.0067
561	Xgla4546	(0)	13	:	0.9931	0	0.0069
562	Xgla4547	(0)	13	:	0.9958	0	0.0042
563	Xgla4548	(0)	13	:	0.9397	0.0179	0.0424
564	Xgla4549	(0)	13	:	0.9655	0	0.0345
565	Xgla4550	(0)	13	:	0.9954	0	0.0046
566	Xgla4551	(0)	13	:	0.9953	0	0.0047
567	Xgla4552	(0)	13	:	0.9947	0.0013	0.004
568	Xgla4553	(0)	13	:	0.9269	0.0314	0.0417
569	Xgla4554	(0)	13	:	0.9953	0.0008	0.0039
570	Xgla4555	(0)	13	:	0.992	0	0.008
571	Xgla4556	(0)	13	:	0.9923	0	0.0077
572	Xgla4557	(0)	13	:	0.996	0.0001	0.0039
573	Xgla4558	(0)	13	:	0.9929	0	0.0071
574	Xgla6767	(0)	13	:	0.9857	0.0054	0.0089
575	Xgla6768	(0)	13	:	0.9957	0	0.0043
576	Xgla6769	(0)	13	:	0.9956	0	0.0044
577	Xgla211	(0)	14	:	0.9458	0.0198	0.0344
578	Xgla217	(0)	14	:	0.9907	0	0.0093
579	Xgla218	(0)	14	:	0.9906	0.0017	0.0077
580	Xgla219	(0)	14	:	0.9937	0	0.0064
581	Xgla226	(0)	14	:	0.9933	0	0.0067
582	Xgla229	(0)	14	:	0.9959	0	0.0041
583	Xgla2887	(0)	14	:	0.9965	0	0.0035
584	Xgla2888	(0)	14	:	0.9932	0	0.0068
585	Xgla2890	(0)	14	:	0.9915	0	0.0086
586	Xgla2891	(0)	14	:	0.9888	0.0012	0.01
587	Xgla2892	(0)	14	:	0.9973	0	0.0027
588	Xgla2893	(0)	14	:	0.993	0.0004	0.0066
589	Xgla2894	(0)	14	:	0.9991	0	0.0009
590	Xgla2895	(0)	14	:	0.9639	0.001	0.0351
591	Xgla2896	(0)	14	:	0.9858	0	0.0142
592	Xgla2898	(0)	14	:	1	0	0
593	Xgla2899	(0)	14	:	0.9945	0	0.0055
594	Xgla2900	(0)	14	:	0.994	0	0.006
595	Xgla2901	(0)	14	:	0.9958	0	0.0042
596	Xgla2902	(0)	14	:	0.997	0	0.0031
597	Xgla2903	(0)	14	:	0.9793	0	0.0207
598	Xgla2904	(0)	14	:	0.9333	0.0019	0.0648
599	Xgla2905	(0)	14	:	0.9863	0.0013	0.0124
600	Xgla2906	(0)	14	:	0.9937	0.0009	0.0054
601	Xgla2907	(0)	14	:	0.9948	0.001	0.0042
602	Xgla2908	(0)	14	:	0.977	0	0.023
603	Xgla2909	(0)	14	:	0.9961	0.0001	0.0038
604	Xgla2910	(0)	14	:	0.9867	0.0001	0.0132
605	Xgla3261	(0)	14	:	0.9935	0	0.0065
606	Xgla3263	(0)	14	:	0.9973	0	0.0027
607	Xgla3264	(0)	14	:	0.9662	0.0126	0.0212
608	Xgla3265	(0)	14	:	0.9918	0.0019	0.0064
609	Xgla3266	(0)	14	:	0.9934	0.001	0.0056
610	Xgla3267	(0)	14	:	0.9572	0.0092	0.0336
611	Xgla3269	(0)	14	:	0.9921	0	0.008
612	Xgla3270	(0)	14	:	0.9937	0	0.0063
613	Xgla3271	(0)	14	:	0.9885	0	0.0115
614	Xgla3272	(0)	14	:	0.9944	0	0.0056
615	Xgla3273	(0)	14	:	0.9428	0.0037	0.0535
616	Xgla3275	(0)	14	:	0.991	0	0.009
617	Xgla3276	(0)	14	:	0.9964	0	0.0036
618	Xgla3277	(0)	14	:	0.9802	0.0026	0.0172
619	Xgla3278	(0)	14	:	0.9889	0	0.0111
620	Xgla3282	(0)	14	:	0.9943	0	0.0057
621	Xgla3283	(0)	14	:	0.998	0	0.002
622	Xgla6218	(0)	15	:	0.9849	0	0.0151
623	Xgla6219	(0)	15	:	0.9894	0	0.0107
624	Xgla6228	(0)	15	:	0.9877	0	0.0123
625	Xgla6233	(0)	15	:	0.8723	0.0409	0.0868
626	Xgla6234	(0)	15	:	0.9882	0.0025	0.0093
627	Xgla6235	(0)	15	:	0.9925	0.001	0.0065
628	Xgla6236	(0)	15	:	0.9925	0	0.0076
629	Xgla6237	(0)	15	:	0.9931	0.001	0.0059
630	Xgla6239	(0)	15	:	0.9829	0	0.0171

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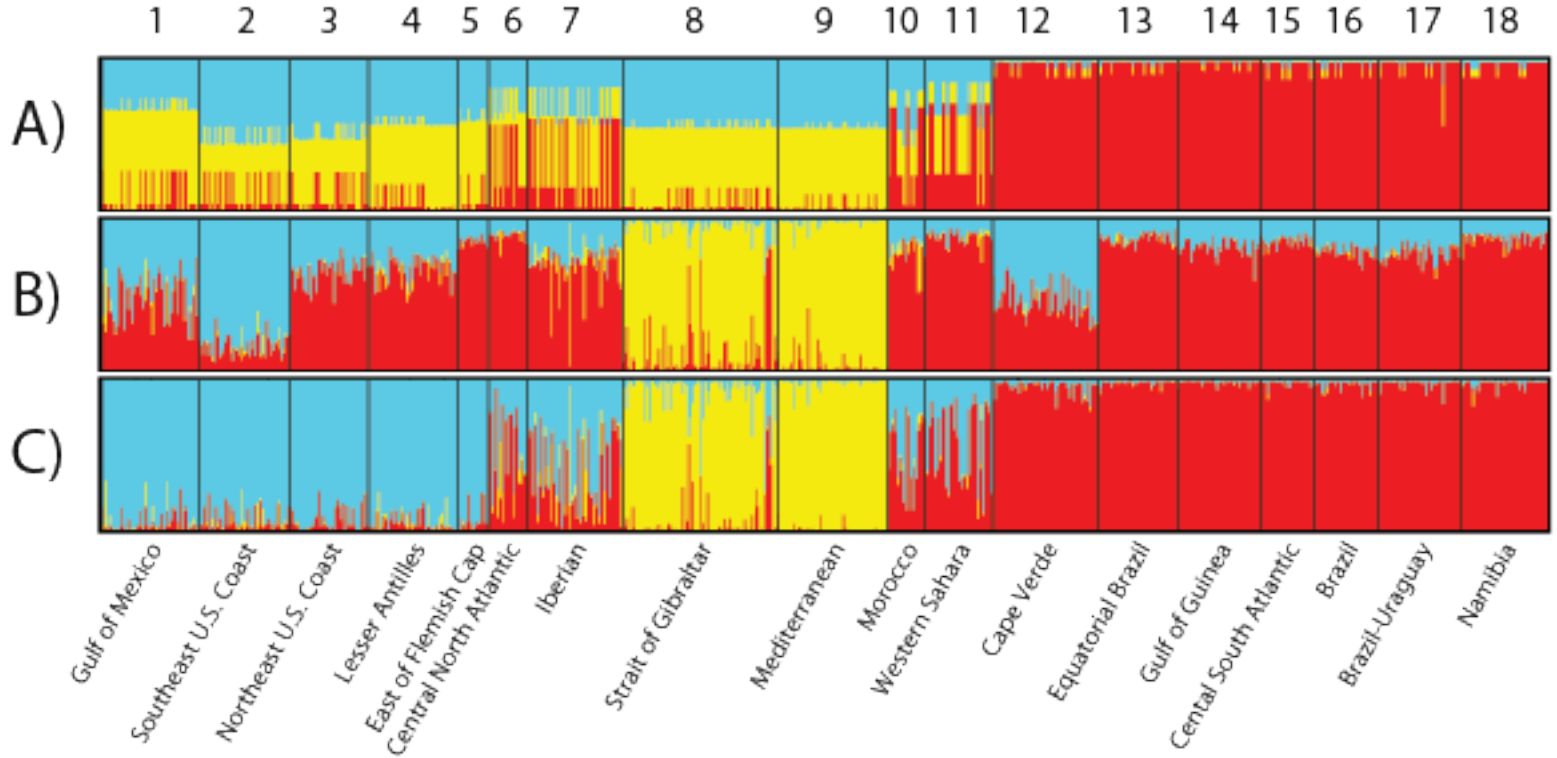
631	Xgla6241	(0)	15	:	0.9874	0.0051	0.0074
632	Xgla6245	(0)	15	:	0.9913	0.0003	0.0084
633	Xgla6249	(0)	15	:	0.9797	0	0.0203
634	Xgla6252	(0)	15	:	0.9971	0	0.0029
635	Xgla6253	(0)	15	:	0.952	0	0.048
636	Xgla6420	(0)	15	:	0.9859	0.0009	0.0132
637	Xgla6421	(0)	15	:	0.9474	0.0003	0.0523
638	Xgla6422	(0)	15	:	0.9919	0	0.0081
639	Xgla6424	(0)	15	:	0.9958	0.0001	0.004
640	Xgla6425	(0)	15	:	0.9916	0.0026	0.0058
641	Xgla6426	(0)	15	:	0.9929	0	0.0071
642	Xgla6427	(0)	15	:	0.9966	0	0.0035
643	Xgla6428	(0)	15	:	0.9892	0	0.0108
644	Xgla6429	(0)	15	:	0.9869	0	0.0131
645	Xgla6430	(0)	15	:	0.9968	0	0.0032
646	Xgla6431	(0)	15	:	0.99	0.0009	0.0091
647	Xgla6432	(0)	15	:	0.9925	0	0.0075
648	Xgla6435	(0)	15	:	0.9543	0.001	0.0447
649	Xgla6439	(0)	15	:	0.9884	0.0063	0.0053
650	Xgla6418	(0)	16	:	0.9654	0.0001	0.0345
651	Xgla6419	(0)	16	:	0.978	0.0119	0.0101
652	Xgla6645	(0)	16	:	0.98	0.0001	0.0199
653	Xgla6647	(0)	16	:	0.9869	0.001	0.0121
654	Xgla6648	(0)	16	:	0.9193	0.0013	0.0795
655	Xgla6649	(0)	16	:	0.897	0.0125	0.0905
656	Xgla7685	(0)	16	:	0.9903	0.0006	0.0091
657	Xgla7686	(0)	16	:	0.9179	0.0309	0.0512
658	Xgla7687	(0)	16	:	0.988	0	0.012
659	Xgla7688	(0)	16	:	0.99	0.0014	0.0086
660	Xgla7689	(0)	16	:	0.9948	0	0.0052
661	Xgla7690	(0)	16	:	0.961	0	0.039
662	Xgla7691	(0)	16	:	0.9874	0	0.0126
663	Xgla7692	(0)	16	:	0.9953	0	0.0047
664	Xgla7693	(0)	16	:	0.9153	0.0021	0.0826
665	Xgla7694	(0)	16	:	0.9924	0	0.0076
666	Xgla7695	(0)	16	:	0.99	0.001	0.009
667	Xgla7696	(0)	16	:	0.9904	0	0.0096
668	Xgla7697	(0)	16	:	0.9838	0.0026	0.0135
669	Xgla7698	(0)	16	:	0.9715	0.0104	0.0181
670	Xgla7699	(0)	16	:	0.9964	0	0.0036
671	Xgla7700	(0)	16	:	0.9775	0.0025	0.0199
672	Xgla7701	(0)	16	:	0.9913	0.0002	0.0085
673	Xgla7702	(0)	16	:	0.9945	0	0.0055
674	Xgla7703	(0)	16	:	0.9652	0	0.0348
675	Xgla7704	(0)	16	:	0.9917	0	0.0084
676	Xgla7705	(0)	16	:	0.8847	0.0008	0.1145
677	Xgla7706	(0)	16	:	0.9808	0	0.0192
678	Xgla7707	(0)	16	:	0.975	0.0019	0.0232
679	Xgla7708	(0)	16	:	0.9936	0	0.0064
680	Xgla7709	(0)	16	:	0.8977	0.0296	0.0727
681	Xgla7710	(0)	16	:	0.9881	0	0.0119
682	Xgla7711	(0)	16	:	0.9917	0	0.0083
683	Xgla7712	(0)	16	:	0.9871	0	0.0129
684	Xgla7713	(0)	16	:	0.9888	0	0.0112
685	Xgla6565	(0)	17	:	0.9877	0.0006	0.0117
686	Xgla6568	(0)	17	:	0.9079	0.0001	0.092
687	Xgla6569	(0)	17	:	0.9948	0	0.0052
688	Xgla6571	(0)	17	:	0.999	0	0.0011
689	Xgla6572	(0)	17	:	0.9834	0.0059	0.0107
690	Xgla6573	(0)	17	:	0.9892	0	0.0108
691	Xgla6574	(0)	17	:	0.9878	0.0026	0.0097
692	Xgla6575	(0)	17	:	0.9893	0.0009	0.0098
693	Xgla6576	(0)	17	:	0.9858	0.0065	0.0077
694	Xgla6577	(0)	17	:	0.9912	0.0015	0.0073
695	Xgla6578	(0)	17	:	0.9913	0.001	0.0077
696	Xgla6579	(0)	17	:	0.9456	0.0034	0.051
697	Xgla6646	(0)	17	:	0.9894	0	0.0106
698	Xgla7634	(0)	17	:	0.9327	0.0114	0.0559
699	Xgla7635	(0)	17	:	0.9916	0	0.0083
700	Xgla7636	(0)	17	:	0.9961	0	0.0039
701	Xgla7637	(0)	17	:	0.9852	0	0.0148

702	Xgla7638	(0)	17	:	0.9947	0	0.0053
703	Xgla7639	(0)	17	:	0.9949	0	0.0051
704	Xgla7640	(0)	17	:	0.9906	0	0.0094
705	Xgla7641	(0)	17	:	0.9952	0.001	0.0038
706	Xgla7642	(0)	17	:	0.9936	0	0.0064
707	Xgla7643	(0)	17	:	0.9882	0.0015	0.0103
708	Xgla7644	(0)	17	:	0.9759	0.001	0.023
709	Xgla7645	(0)	17	:	0.9926	0.0006	0.0068
710	Xgla7646	(0)	17	:	0.8993	0.0029	0.0978
711	Xgla7647	(0)	17	:	0.9956	0	0.0044
712	Xgla7648	(0)	17	:	0.9908	0	0.0092
713	Xgla7649	(0)	17	:	0.9344	0.0009	0.0647
714	Xgla7650	(0)	17	:	0.9808	0	0.0192
715	Xgla7652	(0)	17	:	0.974	0.0106	0.0155
716	Xgla7653	(0)	17	:	0.9857	0	0.0143
717	Xgla7654	(0)	17	:	0.9939	0	0.0061
718	Xgla7655	(0)	17	:	0.9953	0	0.0047
719	Xgla7656	(0)	17	:	0.8411	0.0021	0.1568
720	Xgla7657	(0)	17	:	0.9894	0	0.0106
721	Xgla7658	(0)	17	:	0.9875	0.0022	0.0102
722	Xgla7659	(0)	17	:	0.9904	0	0.0096
723	Xgla7660	(0)	17	:	0.9893	0.0019	0.0088
724	Xgla7661	(0)	17	:	0.9925	0	0.0075
725	Xgla7662	(0)	17	:	0.9913	0.0025	0.0061
726	Xgla7663	(0)	17	:	0.9912	0	0.0088
727	Xgla7664	(0)	17	:	0.9882	0	0.0118
728	Xgla7665	(0)	17	:	0.999	0	0.001
729	Xgla4094	(0)	18	:	0.9587	0.0336	0.0078
730	Xgla4095	(0)	18	:	0.9704	0	0.0296
731	Xgla4096	(0)	18	:	0.9899	0.0023	0.0078
732	Xgla4097	(0)	18	:	0.9938	0	0.0062
733	Xgla4098	(0)	18	:	0.9972	0	0.0028
734	Xgla4099	(0)	18	:	0.9581	0.001	0.0409
735	Xgla4100	(0)	18	:	0.8302	0.0864	0.0834
736	Xgla4101	(0)	18	:	0.9535	0.0019	0.0446
737	Xgla4102	(0)	18	:	0.9642	0.0012	0.0345
738	Xgla4103	(0)	18	:	0.9254	0.0028	0.0718
739	Xgla4104	(0)	18	:	0.9924	0	0.0076
740	Xgla4105	(0)	18	:	0.99	0.0023	0.0077
741	Xgla4106	(0)	18	:	0.9909	0.0031	0.0059
742	Xgla4107	(0)	18	:	0.9936	0	0.0064
743	Xgla4108	(0)	18	:	0.9908	0.0009	0.0083
744	Xgla4109	(0)	18	:	0.9928	0	0.0072
745	Xgla4110	(0)	18	:	0.9845	0.0024	0.013
746	Xgla4111	(0)	18	:	0.9925	0.0007	0.0068
747	Xgla4112	(0)	18	:	0.9936	0.0009	0.0054
748	Xgla4113	(0)	18	:	0.8925	0	0.1075
749	Xgla4114	(0)	18	:	0.9904	0.0007	0.0089
750	Xgla4115	(0)	18	:	0.9872	0.0011	0.0118
751	Xgla4116	(0)	18	:	0.9943	0	0.0057
752	Xgla4117	(0)	18	:	0.9698	0.0107	0.0195
753	Xgla4118	(0)	18	:	0.9967	0	0.0034
754	Xgla4119	(0)	18	:	0.9873	0	0.0127
755	Xgla4120	(0)	18	:	0.9943	0	0.0057
756	Xgla4121	(0)	18	:	0.9892	0	0.0108
757	Xgla4122	(0)	18	:	0.9918	0.0004	0.0078
758	Xgla4123	(0)	18	:	0.9961	0	0.0039
759	Xgla4235	(0)	18	:	0.9804	0.0063	0.0133
760	Xgla4236	(0)	18	:	0.9413	0	0.0587
761	Xgla4237	(0)	18	:	0.9952	0	0.0048
762	Xgla4238	(0)	18	:	0.9774	0	0.0226
763	Xgla4239	(0)	18	:	0.9231	0.001	0.0759
764	Xgla4240	(0)	18	:	0.9947	0.0002	0.0051
765	Xgla4241	(0)	18	:	0.9917	0.001	0.0073
766	Xgla4242	(0)	18	:	0.9919	0	0.0081
767	Xgla4244	(0)	18	:	0.9934	0.0009	0.0056
768	Xgla4245	(0)	18	:	0.9887	0.0007	0.0106
769	Xgla4246	(0)	18	:	0.9882	0	0.0118
770	Xgla4248	(0)	18	:	0.9899	0.0012	0.0089
771	Xgla4249	(0)	18	:	0.9915	0	0.0086
772	Xgla4250	(0)	18	:	0.9895	0	0.0105

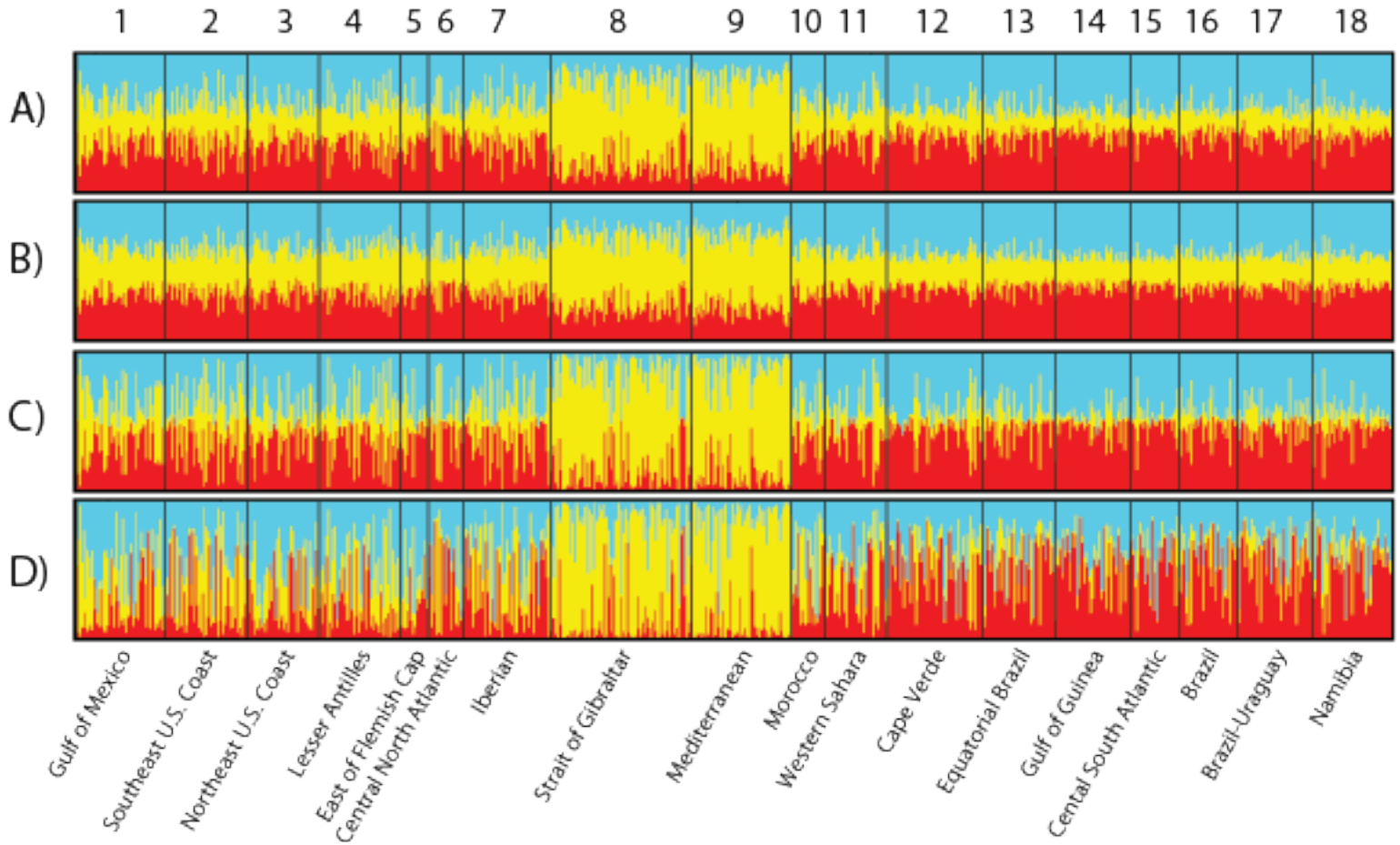
A-14 continued

773	Xgla4252	(0)	18	:	0.9902	0	0.0098
774	Xgla4253	(0)	18	:	0.9919	0	0.0081

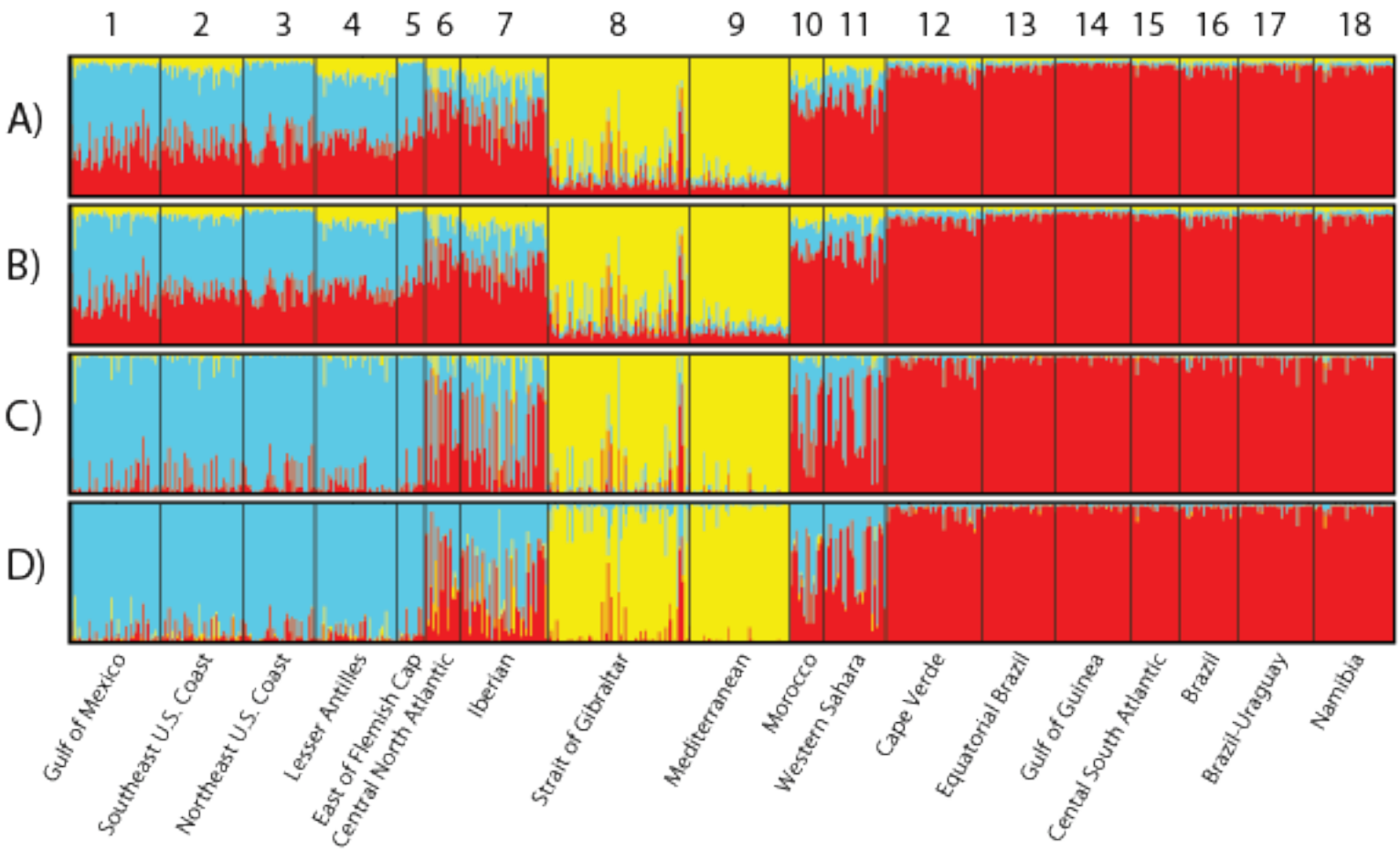
A-15. STRUCTURE v2.3 individual assignments of 774 swordfish from 18 localities using the no admixture and correlated allele model and the location prior with A) only the CaM locus, B) all loci except the CaM locus, and C) all loci including the CaM locus.



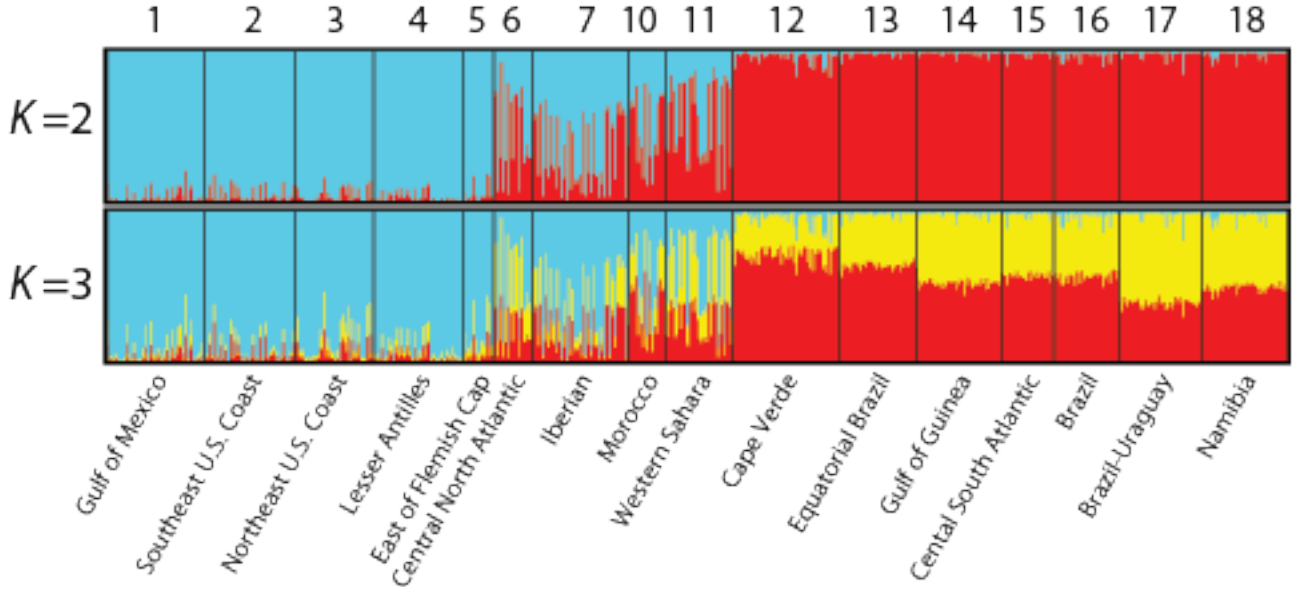
A-16. STRUCTURE v2.3 individual assignments for 774 swordfish from 18 localities using no location prior and the A) admixture and correlated allele model, B) admixture and independent allele model, C) no admixture correlated allele model, and D) no admixture and independent allele model.



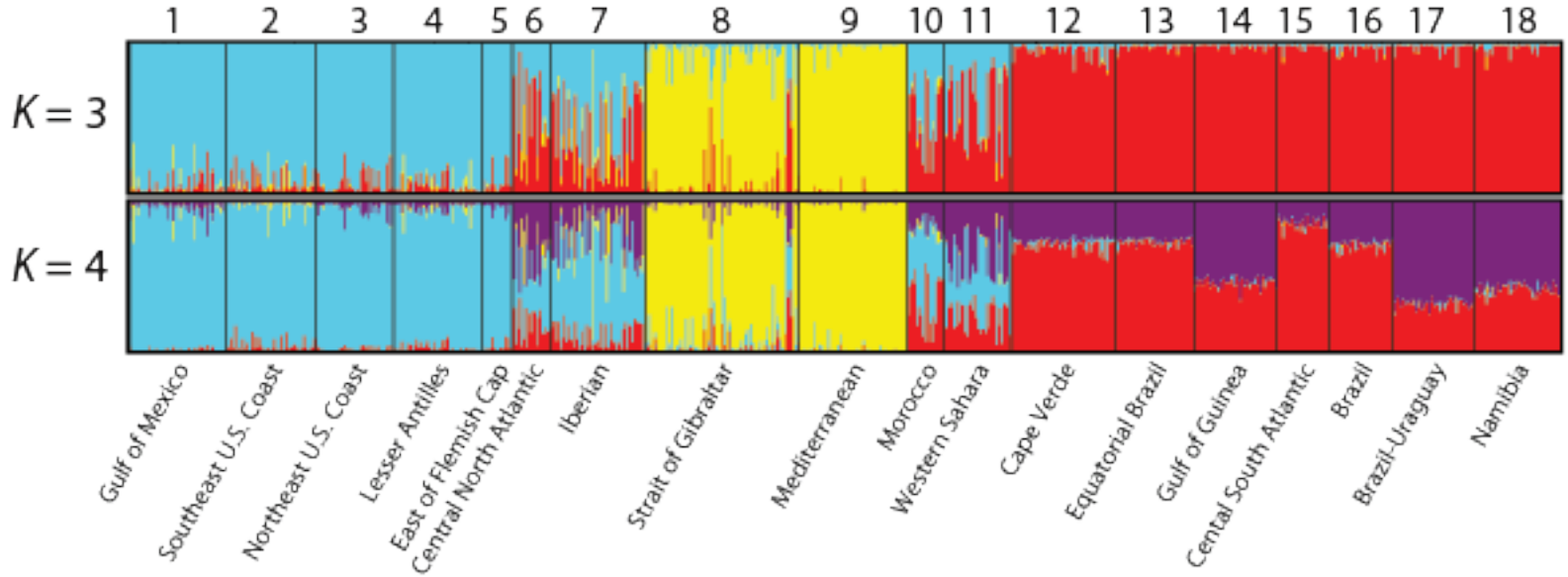
A-17. STRUCTURE v2.3 individual assignments for 774 swordfish from 18 localities using location prior and the A) admixture and independent allele model, B) admixture and correlated allele model, C) no admixture independent allele model, and D) no admixture and correlated allele model.



A-18. STRUCTURE v2.3 independent assignments for 632 swordfish from 16 localities in the North Atlantic and South Atlantic using the no admixture correlated allele model with a location prior for *K*=2 and *K*=3.



A-19. STRUCTURE v2.3 independent assignments for 774 swordfish from 18 localities in the North Atlantic, South Atlantic, and Mediterranean Sea using the no admixture correlated allele model with a location prior for *K*=2 and *K*=3.



A-20. Consensus power of assignment of each locus using WHICHLOCI v.1.0 between North Atlantic (n=207) and Mediterranean (n=142) swordfish.

Rank	Locus	Score	% (Relative Score)
1	Mlc2	0.3059	18.8959
2	ATPsß	0.2611	16.1321
3	Act2a	0.182	11.2466
4	LDHA	0.176	10.8756
5	ANT	0.1713	10.5845
6	ALDB	0.1377	8.5069
7	SRP54	0.1371	8.4701
8	ARP	0.1192	7.3614
9	GpHR	0.0649	4.0112
10	CaM	0.0634	3.9156

A-21. Consensus critical population (South Atlantic) power of assignment of each locus using WHICHLOCI v.1.0 between North Atlantic (n=207) and South Atlantic (n=297) swordfish.

Rank	Locus	Score	% (Relative Score)
1	CaM	-0.71	2.1347
2	Mlc2	-0.72	2.1648
3	SRP54	-1.56	4.6903
4	ALDB	-1.69	5.0812
5	ARP	-3.35	10.0722
6	Act2a	-3.95	11.8761
7	LDHA	-4.83	14.5219
8	GpHR	-5.02	15.0932
9	ATPsß	-5.33	16.0253
10	ANT	-6.1	18.3403